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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 C.F.R. § 1.53(b))

Attorney Docket No.	MBX 030
First Inventor or Application Identifier	Oliver P. Peoples
Title	ENZYMES FOR BIOPOLYMER PRODUCTION
Express Mail Label No.	EL 320 553 823 US

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

ADDRESS TO: **Assistant Commissioner for Patents, Box Patent Application, Washington, DC 20231**

1. ☒ * Fee Transmittal Form *(e.g., PTO/SB/17)*
   (Submit an original and a duplicate for fee processing)
2. ☒ Specification [*Total Pages* 32]
   (preferred arrangement set forth below)
   - Descriptive title of the Invention
   - Cross References to Related Applications
   - Statement Regarding Fed sponsored R & D
   - Reference to Microfiche Appendix
   - Background of the Invention
   - Brief Summary of the Invention
   - Brief Description of the Drawings *(if filed)*
   - Detailed Description
   - Claim(s)
   - Abstract of the Disclosure
3. ☒ Drawing(s) *(35 U.S.C. 113)* [*Total Sheets* 2]
4. Oath or Declaration [*Total Pages*]
   a. ☒ Unexecuted
   b. ☐ Copy from a prior application (37 C.F.R. § 1.63(d))
   (for continuation/divisional with Box 16 completed)
   i. ☐ DELETION OF INVENTOR(S)
   Signed statement attached deleting inventor(s) named in the prior application, see 37 C.F.R. §§ 1.63(d)(2) and 1.33(b).

** NOTE FOR ITEMS 1 & 13: IN ORDER TO BE ENTITLED TO PAY SMALL ENTITY FEES, A SMALL ENTITY STATEMENT IS REQUIRED (37 C.F.R. § 1.27), EXCEPT IF ONE FILED IN A PRIOR APPLICATION IS RELIED UPON (37 C.F.R. § 1.28).*

5. ☐ Microfiche Computer Program *(Appendix)*
6. Nucleotide and/or Amino Acid Sequence Submission *(if applicable, all necessary)*
   a. ☑ Computer Readable Copy
   b. ☐ Paper Copy (identical to computer copy)
   c. ☑ Statement verifying identity of above copies

ACCOMPANYING APPLICATION PARTS

7. ☐ Assignment Papers (cover sheet & document(s))
8. ☐ 37 C.F.R.§3.73(b) Statement *(when there is an assignee)* ☐ Power of Attorney
9. ☐ English Translation Document *(if applicable)*
10. ☐ Information Disclosure Statement (IDS)/PTO-1449 ☐ Copies of IDS Citations
11. ☐ Preliminary Amendment
12. ☒ Return Receipt Postcard (MPEP 503) *(Should be specifically itemized)*
13. ☒ * Small Entity Statement(s) *(PTO/SB/09-12)* ☐ Statement filed in prior application, Status still proper and desired
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STATEMENT CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) & 1.27(c))--SMALL BUSINESS CONCERN	Docket Number (Optional) MBX 030

Applicant, Patentee, or Identifier: Oliver P. Peoples, Lara Madison, Gjalt Huisman
Application or Patent No.:
Filed or Issued: July 30, 1999
Title. ENZYMES FOR BIOPOLYMER PRODUCTION

I hereby state that I am
☐ the owner of the small business concern identified below:
☒ an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF SMALL BUSINESS CONCERN Metabolix, Inc.

ADDRESS OF SMALL BUSINESS CONCERN 303 Third Street
Cambridge, Massachusetts 02142-1196

I hereby state that the above identified small business concern qualifies as a small business concern as defined in 13 CFR Part 121 for purposes of paying reduced fees to the United States Patent and Trademark Office. Questions related to size standards for a small business concern may be directed to: Small Business Administration, Size Standards Staff, 409 Third Street, SW, Washington, DC 20416

I hereby state that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention described in:

☐ the specification filed herewith with title as listed above.
☒ the application identified above.
☐ the patent identified above.

If the rights held by the above identified small business concern are not exclusive, each individual, concern, or organization having rights in the invention must file separate statements as to their status as small entities, and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d), or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern, or organization having any rights in the invention is listed below:
☒ no such person, concern, or organization exists.
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NAME OF PERSON SIGNING Oliver P. Peoples

TITLE OF PERSON IF OTHER THAN OWNER Vice President, Research and Development

ADDRESS OF PERSON SIGNING 27 Radcliffe Road, Arlington, Massachusetts 02174

SIGNATURE Oliver P. Peoples DATE 7/30/99

APPLICATION

FOR

UNITED STATES LETTERS PATENT

BY

OLIVER P. PEOPLES

LARA L. MADISON

AND

GJALT W. HUISMAN

FOR

ENZYMES FOR BIOPOLYMER PRODUCTION

ENZYMES FOR BIOPOLYMER PRODUCTION

Cross-Reference To Related Applications

Priority is claimed to U.S. provisional application Serial No. 60/094,674, filed July 30, 1998.

Background Of The Invention

The present invention is generally in the field of genetically engineered bacterial and plant systems for production of polyhydroxyalkanoates by microorganisms and genetically engineered plants, wherein the enzymes essential for production of the polymers are expressed as fusion proteins having enhanced properties for polymer synthesis.

Numerous microorganisms have the ability to accumulate intracellular reserves of poly[(*R*)-3-hydroxyalkanoate] polymers or PHAs. PHAs are biodegradable and biocompatible thermoplastic materials with a broad range of industrial and biomedical applications (Williams and Peoples, 1996, CHEMTECH 26, 38-44). In recent years, the PHA biopolymers have emerged from what was originally considered to be a single homopolymer, poly-3-hydroxybutyrate (PHB), into a broad class of polyesters with different monomer compositions and a wide range of physical properties. Over 100 different monomers have been incorporated into the PHA polymers (Steinbüchel and Valentin, 1995, FEMS Microbiol. Lett. 128; 219-228). It has been useful to divide the PHAs into two groups according to the length of their side chains and their biosynthetic pathways. Those with short side chains, such as polyhydroxybutyrate (PHB), a homopolymer of *R*-3-hydroxybutyric acid units, are semi-crystalline thermoplastics, whereas PHAs with long side chains are more elastomeric.

Biosynthesis of the short side-chain PHAs such as PHB and PHBV proceeds through a sequence of three enzyme catalyzed reactions from the central metabolite acetyl-CoA. In the first step of this pathway, two acetyl-CoA

molecules are condensed to acetoacetyl-CoA by a 3-ketoacyl-CoA thiolase. Acetoacetyl-CoA is subsequently reduced to the PHB precursor 3-hydroxybutyryl-CoA by an NADPH dependent reductase. 3-hydroxybutyryl-CoA is then polymerized to PHB which is sequestered by the bacteria as "intracellular inclusion bodies" or granules. The molecular weight of PHB is generally in the order of 10^4-10^7 Da. In some bacteria such as *Chromatium vinosum* the reductase enzyme is active primarily with NADH as co-factor. The synthesis of the PHBV co-polymer proceeds through the same pathway, with the difference being that acetyl-CoA and propionyl-CoA are converted to 3-ketovaleryl-CoA by ß-ketothiolase. 3-ketovaleryl-CoA is then converted to 3-hydroxyvaleryl-CoA which is polymerized.

Long side chain PHAs are produced from intermediates of fatty acid ß-oxidation or fatty acid biosynthesis pathways. In the case of ß-oxidation, the L-isomer of ß-hydroxyacyl-CoA is converted to the D-isomer by an epimerase activity present on the multi-enzyme complex encoded by the *fao*AB genes. Biosynthesis from acetyl-CoA through the fatty acid synthase route produces the L-isomer of ß-hydroxyacyl-ACP. Conversion of the ACP to the CoA derivative is catalyzed by the product of the *pha*G gene (Kruger and Steinbuchel 1998, U.S. patent 5,750,848).

Enoyl-CoA hydratases have been implicated in PHA biosynthesis in microbes such as *Rhodospirillum rubrum* and *Aeromonas caviae*. The biosynthesis of PHB in *R. rubrum* is believed to proceed through an acetoacetyl-CoA reductase enzyme specific for the L-isomer of 3-hydroxybutyryl-CoA. Conversion of the L to the D form is then catalysed by the action of two enoyl-CoA hydratase activities. In the case of the PHB-co-HX, where X is a C6-C16 hydroxy acid, copolymers which are usually produced from cells grown on fatty acids, a combination of these routes can be responsible for the formation of the different monomeric units. Indeed, analysis of the DNA locus encoding the PHA synthase gene in *Aeromonas caviae,* which produces the copolymer PHB-co-3-hydroxyhexanoate, identified a gene encoding a D-

specific enoyl-CoA hydratase responsible for the production of the D-ß-hydroxybutyryl-CoA and D-ß-hydroxyhexanoyl-CoA units (Fukui and Doi, 1997, J. Bacteriol. 179: 4821-4830; Fukui et. al., 1998, J. Bacteriol. 180: 667-673).

It is desirable for economic reasons to be able to produce these polymers in transgenic crop species. Methods for achieving this are known. See, for example, U.S. patent NO. 5 ,245,023 and U.S. patent NO. 5 ,250,430; U.S. patent NO. 5 ,502,273; U.S. patent NO. 5 ,534,432; U.S. patent NO. 5 ,602,321; U.S. patent NO. 5,610,041; U.S. patent NO. 5 ,650,555: U.S. patent NO. 5 ,663,063; WO, 9100917, WO 9219747, WO 9302187, WO 9302194 and WO 9412014, Poirier et.al., 1992, Science 256; 520-523, Williams and Peoples, 1996, Chemtech 26, 38-44. In order to achieve this goal, it is necessary to transfer a gene, or genes in the case of a PHA synthase with more than one subunit, encoding a PHA synthase from a microorganism into plant cells and obtain the appropriate level of production of the PHA synthase enzyme. In addition it may be necessary to provide additional PHA biosynthetic genes, eg. a ketoacyl-CoA thiolase, an acetoacetyl-CoA reductase gene, a 4-hydroxybutyryl-CoA transferase gene or other genes encoding enzymes required to synthesize the substrates for the PHA synthase enzymes.

In many cases, it is particularly desirable to control the expression in different plant tissues or organelles. Methods for controlling expression are known to those skilled in the art (Gasser and Fraley, 1989, Science 244; 1293-1299; Gene Transfer to Plants,1995, Potrykus, I. and Spangenberg, G. eds. Springer -Verlag Berlin Heidelberg New York. and "Transgenic Plants: A Production System for Industrial and Pharmaceutical Proteins", 1996, Owen, M.R.L. and Pen, J. Eds. John Wiley & Sons Ltd. England). U.S. PATENT NO. 5 ,610,041 describes the route of plastid expression by the previously known technology of adding a leader peptide to direct the protein expressed from the nuclear gene to the plastid. More recent technology enables the direct insertion of foreign genes directly into the plastid chromosome by

recombination (Svab *et al.*, 1990, Proc. Natl. Acad. Sci. USA. 87: 8526-8530; McBride et al., 1994, Proc. Natl. Acad. Sci. USA. 91: 7301-7305). The prokaryotic nature of the plastid RNA and protein synthesis machinery also allows for the expression of microbial genes such as for example the *phbC*, *phbA* and *phbB* genes of *R. eutropha.*

Genetic engineering of bacteria and plants to make products such as polymers which require the coordinated expression and action of multiple enzymes, sequentially on different substrates, may result in low yields, or poor efficiencies, or variations or deviation in the final product.

It is therefore an object of the present invention to provide methods and materials for enhancing production of products of multiple enzymes, such as polymers, and particularly polyhydroxyalkanoates, in bacteria or plants.

Summary of the Invention

In order to optimize the flux or flow of carbon intermediates from normal cellular metabolism into PHAs it is desirable to optimize the expression of the enzymes of the PHA biosynthetic pathway. Gene fusions are genetic constructs where two open reading frames have been fused into one. The transcriptional and translational sequences upstream of the first open reading frame direct the synthesis of a single protein with the primary structure that comprises both original open reading frames. Consequently, gene fusions encode hybrid proteins and in some cases bifunctional hybrid enzymes. Individual genes are isolated, for example, by PCR, such that the resulting DNA fragments contain the complete coding region or parts of the coding region of interest. The DNA fragment that encodes the amino-terminal domain of the hybrid protein may contain a translation initiation site and a transcriptional control sequence. The stop codon in the gene encoding the amino-terminal domain needs to be removed from this DNA fragment. The stop codon in the gene encoding the carboxy-terminal domain needs to be retained in the DNA fragment. DNA sequences that are recognized by restriction enzymes may be introduced into the new genes for DNA cloning purposes. Linkers may be

added to spatially separate the two domains of the hybrid protein.

In the case of enzymes which catalyse successive reactions in a pathway, the fusion of two genes results in bringing two enzymatic activities into close proximity to each other. When the product of the first reaction is a substrate for the second one, this new configuration of active sites may result in a faster transfer of the product of the first reaction to the second active site with a potential for increasing the flux through the pathway. The configuration of the two catalytic domains in the hybrid in relation to one another, may be altered by providing a linker sequence between them. This linker may be composed of any of the twenty natural amino acids and can be of variable length. The variation in length and composition are important parameters for changing the relative configuration of the individual domains of the hybrid and its enzyme activities.

This technology allows for the direct incorporation of a series of genes encoding a multi-enzyme pathway into a bacteria or plant or plant organelle, for example, the plastid genome. In some cases it may be useful to re-engineer the 5'-untranslated regions of plastid genes which are important for mRNA stability and translation (Hauser et al., 1996. J. Biol. Chem. 271: 1486-1497), remove secondary structure elements, or add elements from highly expressed plastid genes to maximize expression of transgenes encoded by an operon.

Examples demonstrate the expression of active polypeptides encoding multiple enzyme activies. These are homotetrameric enzymes which require the use of cofactors and which interact to synthesize polymer, which have not previously been demonstrated to be expressable as fusion proteins.

Brief Description of the Drawings

Figures 1A-1H are schematics of gene fusions encoding multiple-enzyme proteins: pTrcAB including beta-ketothiolase (phbA) and acyl-CoA reductase (phbB) (1A); pTrcBA including phbB and phbA (1B); pTrcCP

including PHA synthase (phaC) and phasin (phaP) (1C); pTrcPC including phaP and phaC (1D); pTrcCG including phaC and beta-hydroxyacyl-ACP::coenzyme-A transferase (phbG) (1E); pTrcGC including phbG and phaC (1F); pTrcCJ including phaC and enoyl-CoA hydratases (phaJ) (1G); and pTrcJC including phaJ and phaC (1H).

Figure 2 is a schematic of the construction of pTrcAB11, including phbA and phbB, on a single polypeptide with both thiolase and reductase activity.

Detailed Description of the Invention

I. Gene Fusions

In order to optimize the flux or flow of carbon intermediates from normal cellular metabolism into PHAs it is desirable to optimize the expression of the enzymes of the PHA biosynthetic pathway. Gene fusions are genetic constructs where two open reading frames have been fused into one. The transcriptional and translational sequences upstream of the first open reading frame direct the synthesis of a single protein with the primary structure that comprises both original open reading frames. Consequently, gene fusions encode hybrid proteins and in some cases bifunctional hybrid enzymes. Hybrid proteins have been developed for applications such as protein purification (Bülow, L., Eur. J. Biochem. (1987) 163: 443-448; Bülow, L., Biochem. Soc. Symp. (1990) 57: 123-133); Bülow, L., Tibtech.(1991) 9: 226-231), biochemical analyses (Ljungcrantz et al. FEBS Lett. (1990) 275: 91-94; Ljungcrantz et al., Biochemistry (1989) 28: 8786-8792; Bülow, L., Biochem. Soc. Symp. (1990) 57: 123-133); Bülow, L., Tibtech.(1991) 9: 226-231) and metabolic engineering (U.S. Patent 5,420, 027; Carlsson, Biotech. Lett. (1992) 14: 439-444; Bülow, L., Biochem. Soc. Symp. (1990) 57: 123-133); Bülow, L., Tibtech.(1991) 9: 226-231; Fisher, Proc. Natl. Acad. Sci. U.S.A. (1992) 89: 10817-10821).

Individual genes are isolated, for example, by PCR, such that the resulting DNA fragments contain the complete coding region or parts of the

coding region of interest. The DNA fragment that encodes the amino-terminal domain of the hybrid protein may contain a translation initiation site and a transcriptional control sequence. The stop codon in the gene encoding the amino-terminal domain needs to be removed from this DNA fragment. The stop codon in the gene encoding the carboxy-terminal domain needs to be retained in the DNA fragment. DNA sequences that are recognized by restriction enzymes may be introduced into the new genes for DNA cloning purposes. Linkers may be added to spatially separate the two domains of the hybrid protein.

In the case of enzymes which catalyse successive reactions in a pathway, the fusion of two genes results in bringing two enzymatic activities into close proximity to each other. When the product of the first reaction is a substrate for the second one, this new configuration of active sites may result in a faster transfer of the product of the first reaction to the second active site with a potential for increasing the flux through the pathway. The configuration of the two catalytic domains in the hybrid in relation to one another, may be altered by providing a linker sequence between them. This linker may be composed of any of the twenty natural amino acids and can be of variable length. The variation in length and composition are important parameters for changing the relative configuration of the individual domains of the hybrid and its enzyme activities.

Methods exist for improving the utility of PHA biosynthetic fusion enzymes using molecular evolution or "gene-shuffling" techniques (Stemmer, M.P.C. 1994, Nature, 370: 389-391; Stemmer, M.P.C. 1994, Proc. Natl. Acad. Sci., 1994, 91: 10747-10751). Requirements to make this approach work include the mutagenesis techniques, which are usually PCR-based, and a screening technique to identify those mutant enzymes with the desired improved properties.

A. Genes

Suitable genes include PHB and PHA synthases, ß-ketothiolases, acyl-CoA reductases, phasins, enoyl-CoA hydratases and ß-hydroxyacyl-ACP::coenzyme-A transferases. Examples of fusions that can be constructed are illustrated in Figures 1A-1H.

ß-ketothiolase encoding genes have been isolated from *Alcaligenes latus* (MBX unpublished; Choi, et al. Appl. Environ. Micrbiol. 64 (12), 4897-4903 (1998)], *Ralstonia eutropha* [Peoples, O.P. and Sinskey, A.J., J. Biol. Chem. 264: 15298-15303 (1989); Slater et. al., 1998, J. Bacteriol. 180: 1979-1987], *Acinetobacter* sp. [Schembri, et al. J. Bacteriol. , *Chromatium vinosum* [Liebergesell, M. and Steinbuchel, A. Eur. J. Biochem. 209 (1), 135-150 (1992)], *Pseudomonas acidophila* (Umeda, et al. Appl. Biochem. Biotech. 70-72: 341-352 (1998)], *Pseudomonas denitrificans* [Yabutani, et al. FEMS Microbiol. Lett. 133 (1-2), 85-90 (1995)], *Rhizobium meliloti* [Tombolini, et al. Microbiology 141, 2553-2559 (1995)], *Thiocystis violacea* [Liebergesell, et al. Appl. Microbiol. Biotechnol. 38 (4), 493-501 (1993)], and *Zoogloea ramigera* [Peoples, et al. J. Biol. Chem. 262 (1), 97-102 (1987)].

Reductase encoding genes have been isolated from *Alcaligenes latus* (Choi, et al. Appl. Environ. Micrbiol. 64 (12), 4897-4903 (1998)], *R. eutropha* [Peoples, O.P. and Sinskey, A.J., J. Biol. Chem. 264 (26), 15298-15303 (1989); *Acinetobacter* sp. (Schembri, et al. J. Bacteriol), *C. vinosum* [Liebergesell, M. and Steinbuchel, A. Eur. J. Biochem. 209 (1), 135-150 (1992)], *Pseudomonas acidophila* (Umeda, et al. Appl. Biochem. Biotech. 70-72: 341-352 (1998)], *P. denitrificans* [Yabutani, et al. FEMS Microbiol. Lett. 133 (1-2), 85-90 (1995)], *R. meliloti* [Tombolini, et al. Microbiology 141 (Pt 10), 2553-2559 (1995)], and *Z. ramigera* [Peoples, O.P. and Sinskey, A.J., 1989, Molecular Microbiology, 3: 349-357).

PHA synthase encoding genes have been isolated from *Aeromonas caviae* [Fukui, T. and Doi, Y. J. Bacteriol. 179 (15), 4821-4830 (1997)], *Alcaligenes latus* (Choi, et al. Appl. Environ. Microbiol. 64 (12), 4897-4903

(1998)], *R. eutropha* [Peoples, O.P. and Sinskey, A.J. J. Biol. Chem. 264 (26), 15298-15303 (1989); Lee, et al. *Acinetobacter* [Schembri, et al. J. Bacteriol.], *C. vinosum* [Liebergesell, M. and Steinbuchel, A. Eur. J. Biochem. 209 (1), 135-150 (1992)], *Methylobacterium extorquens* [Valentin, and Steinbuchel, Appl. Microbiol. Biotechnol. 39 (3), 309-317 (1993)], *Nocardia corallina* (GenBank Acc. No. AF019964), *Nocardia salmonicolor*, *Pseudomonas acidophila* (Umeda, et al. T. Appl. Biochem. Biotech. 70-72: 341-352 (1998)], *P. denitrificans* [Ueda, et al. J. Bacteriol. 178 (3), 774-779 (1996)], *Pseudomonas aeruginosa* [Timm, and Steinbuchel, Eur. J. Biochem. 209 (1), 15-30 (1992)], *Pseudomonas oleovorans* [Huisman, et al. J. Biol. Chem. 266 (4), 2191-2198 (1991)], *Rhizobium etli* [Cevallos, et al. J. Bacteriol. 178 (6), 1646-1654 (1996)], *R. meliloti* [Tombolini, et al. Microbiology 141 (Pt 10), 2553-2559 (1995)], *Rhodococcus ruber* [Pieper, U. and Steinbuechel, A. FEMS Microbiol.Lett. 96 (1), 73-80 (1992)], *Rhodospirrilum rubrum* [Hustede, et al. FEMS Microbiol. Lett. 93, 285-290 (1992)], *Rhodobacter sphaeroides* [Steinbüchel, et al. FEMS Microbiol. Rev. 9 (2-4), 217-230 (1992); Hustede, et al. Biotechnol. Lett. 15, 709-714 (1993)], *Synechocystis* sp. [Kaneko, T., DNA Res. 3 (3), 109-136 (1996)], *T. violaceae* [Liebergesell, et al. Appl. Microbiol. Biotechnol. 38 (4), 493-501 (1993)], and *Z. ramigera* (GenBank Acc. No. U66242).

Other genes that have not been implicated in PHA formation but which share significant homology with the *phb* genes and/or the corresponding gene products may be used as well. Genes encoding thiolase and reductase like enzymes have been identified in a broad range of non-PHB producing bacteria. *E. coli* (U29581, D90851, D90777), *Haemophilus influenzae* (U32761), *Pseudomonas fragi* (D10390), *Pseudomonas aeruginosa* (U88653), *Clostridium acetobutylicum* (U08465), *Mycobacterium leprae* (U00014), *Mycobacterium tuberculosis* (Z73902), *Helicobacter pylori* (AE000582), *Thermoanaerobacterium thermosaccharolyticum* (Z92974), *Archaeoglobus fulgidus* (AE001021), *Fusobacterium nucleatum* (U37723), *Acinetobacter*

calcoaceticus (L05770), *Bacillus subtilis* (D84432, Z99120, U29084) and *Synechocystis* sp. (D90910) all encode one or more thiolases from their chromosome. Eukaryotic organisms such as *Saccharomyces cerevisiae* (L20428), *Schizosaccharomyces pombe* (D89184), *Candida tropicalis* (D13470), *Caenorhabditis elegans* (U41105), human (S70154), rat (D13921), mouse (M35797), radish (X78116), pumpkin (D70895) and cucumber (X67696) also express proteins with significant homology to the 3-ketothiolase from *R. eutropha*.

Genes with significant homology to the *phbB* gene encoding acetoacetyl CoA reductase have been isolated from several organisms: *Azospirillum brasiliense* (X64772, X52913) and *Rhizobium* sp. (U53327, Y00604), *E. coli* (D90745), *Vibrio harveyi* (U39441), *H. influenzae* (U32701), *B. subtilis* (U59433), *P. aeruginosa* (U91631), *Synechocystis* sp. (D90907), *H. pylori* (AE000570), *Arabidopsis thaliana* (X64464), *Cuphea lanceolata* (X64566) and *Mycobacterium smegmatis* (U66800).

A number of proteins which bind to PHA granules have been identified and their genes cloned (Steinbuchel et. al., 1995, Can. J. Microbiol. (Supplement 1) 41:94-105). The current hypothesis is that these proteins play a role similar to the oleosin oil storage proteins (Huang, A.H.C. 1992, Annu. Rev. Plant Physiol. Plant Mol. Biol. 43: 177-200) in oilseeds and have been named phasins. For example, protein GA24 is a 24 kilodalton protein found in PHA producing cells of *Alcaligenes eutrophus* (Wieczorek et al., J. Bacteriol. 1995, 177, 2425-2435). The gene encoding GA24, *phaP*, has been isolated by complementation of PHA-leaky mutants of the bacterium. Wieczorek et al., in their studies of GA24, observed that the protein coated PHA granules in PHA producing cells of *A. eutrophus*, and that cells deficient in GA24 formed very large granules whereas wild-type cells possessed much smaller granules (Wieczorek et al., J. Bacteriol. 1995, 177, 2425-2435). Based on this observation, the authors proposed that GA24 is one of a number of such proteins termed phasins responsible for controlling PHA granule size. An

immunological analysis of other PHA granules from a number of different bacteria indicated conservation of this protein (Wieczorek et. al., 1996, FEMS Microbiology letters 135: 23-30) and the authors concluded that homologs to GA24 are widespread and their genes can be readily isolated. A 13Kd phasin has been identified in *Acinetobacter sp.* (Schembri et. al., 1995, FEMS Micro. Lett. 133: 277-283).

B. Transformation Vectors

DNA constructs include transformation vectors capable of introducing transgenes into plants. There are many plant transformation vector options available. See (Gene Transfer to Plants (1995), Potrykus, I. and Spangenberg, G. eds. Springer -Verlag Berlin Heidelberg New York; "Transgenic Plants: A Production System for Industrial and Pharmaceutical Proteins" (1996), Owen, M.R.L. and Pen, J. eds. John Wiley & Sons Ltd. England and Methods in Plant Molecular Biology-a laboratory course manual (1995), Maliga, P., Klessig, D.F., Cashmore, A. R., Gruissem, W. and Varner, J.E. eds. Cold Spring Laboratory Press, New York).

C. Regulatory Sequences

In general, plant transformation vectors comprise one or more coding sequences of interest under the transcriptional control of 5' and 3' regulatory sequences, including a promoter, a transcription termination and/or polyadenylation signal and a selectable or screenable marker gene. The usual requirements for 5' regulatory sequences include a promoter, a transcription initiation site, and a mRNA processing signal. 3' regulatory sequences include a transcription termination and/or a polyadenylation signal. Additional RNA processing signals and ribozyme sequences can be engineered into the construct for the expression of two or more polypeptides from a single transcript (U.S. PATENT NO. 5 ,519,164). This approach has the advantage of locating multiple transgenes in a single locus which is advantageous in subsequent plant breeding efforts. An additional approach is to use a vector to specifically transform the plant plastid chromosome by homologous recombination (US

5,545,818), in which case it is possible to take advantage of the prokaryotic nature of the plastid genome and insert a number of transgenes as an operon.

A large number of plant promoters are known and result in either constitutive, or environmentally or developmentally regulated expression of the gene of interest. Plant promoters can be selected to control the expression of the transgene in different plant tissues or organelles, as described by (Gasser and Fraley, 1989, Science 244; 1293-1299). The 5' end of the transgene may be engineered to include sequences encoding plastid or other subcellular organelle targeting peptides linked in-frame with the transgene. Suitable constitutive plant promoters include the cauliflower mosaic virus 35S promoter (CaMV) and enhanced CaMV promoters (Odell et. al., 1985, Nature, 313: 810), actin promoter (McElroy et al., 1990, Plant Cell 2: 163-171), AdhI promoter (Fromm et. al., 1990, Bio/Technology 8: 833-839; Kyozuka et al., 1991, Mol. Gen. Genet. 228: 40-48), ubiquitin promoters, the Figwort mosaic virus promoter, mannopine synthase promoter, nopaline synthase promoter and octopine synthase promoter. Useful regulatable promoter systems include spinach nitrate-inducible promoter, heat shock promoters, small subunit of ribulose biphosphate carboxylase promoters and chemically inducible promoters (U.S. Patent NO. 5 ,364,780 and U.S. Patent NO. 5 ,364,780).

It may be preferable to express the transgenes only in the developing seeds. Promoters suitable for this purpose include the napin gene promoter (U.S. PATENT NO. 5 ,420,034; U.S. PATENT NO. 5 ,608,152), the acetyl-CoA carboxylase promoter (U.S. PATENT NO. 5 ,420,034; U.S. PATENT NO. 5 ,608,152), 2S albumin promoter, seed storage protein promoter, phaseolin promoter (Slightom et. al., 1983, Proc. Natl. Acad. Sci. USA 80: 1897-1901), oleosin promoter (plant et. al., 1994, Plant Mol. Biol. 25: 193-205; Rowley et. al., 1997, Biochim. Biophys. Acta.1345: 1-4; U.S. PATENT NO. 5 ,650,554; PCT WO 93/20216), zein promoter, glutelin promoter, starch synthase promoter, and starch branching enzyme promoter.

A number of useful plant vectors comprising many of the features

described above have been described in the literature. Particularly useful among these are the "super-binary" vectors described by Ishida et. al., (1996, Nature biotechnology 14: 745-750) and the extensive range of vectors available from Cambia, Canberra, Australia (described by Roberts et. al., "A comprehensive set of modular vectors for advanced manipulations and efficient transformation of plants" presented at the Rockefeller Foundation Meeting of the International Program on Rice Biotechnology, 15-18 September 1997, Malacca, Malaysia).

II. Methods for Transformation of Plants and Selection Thereof

It is preferable to express more than one gene product in the plant. A number of methods can be used to achieve this including: introducing the encoding DNAs in a single transformation event where all necessary DNAs are on a single vector; in a co-transformation event where all necessary DNAs are on separate vectors but introduced into plant cells simultaneously; introducing the encoding DNAs by independent transformation events successively into the plant cells i.e. transformation of transgenic plant cells expressing one or more of the encoding DNAs with additional DNA constructs; transformation of each of the required DNA constructs by separate transformation events, obtaining transgenic plants expressing the individual proteins and using traditional plant breeding methods to incorporate the entire pathway into a single plant.

The transformation of suitable agronomic plant hosts using these vectors can be accomplished by a range of methods and plant tissues. Suitable plants include: the *Brassica* family including *napus, rappa, sp. carinata* and *juncea*, maize, soybean, cottonseed, sunflower, palm, coconut, safflower, peanut, mustards including *Sinapis alba* and flax. Suitable tissues for transformation using these vectors include protoplasts, cells, callus tissue, leaf discs, pollen, meristems etc. Suitable transformation procedures include *Agrobacterium*-mediated transformation, biolistics, microinjection, electroporation, polyethylene glycol-mediated protoplast transformation, liposome-mediated transformation, silicon fiber-mediated transformation (U.S. PATENT NO. 5 ,464,765) etc. (Gene Transfer to Plants (1995), Potrykus, I. and Spangenberg,

G. eds. Springer -Verlag Berlin Heidelberg New York; "Transgenic Plants: A Production System for Industrial and Pharmaceutical Proteins" (1996), Owen, M.R.L. and Pen, J. eds. John Wiley & Sons Ltd. England and Methods in Plant Molecular Biology-a laboratory course manual (1995), Maliga, P., Klessig, D.F., Cashmore, A. R., Gruissem, W. and Varner, J.E. eds. Cold Spring Laboratory Press, New York).

Transformation procedures have been established for these specific crops (Gene Transfer to Plants (1995), Potrykus, I. and Spangenberg, G. eds. Springer -Verlag Berlin Heidelberg New York; "Transgenic Plants: A Production System for Industrial and Pharmaceutical Proteins" (1996), Owen, M.R.L. and Pen, J. eds. John Wiley & Sons Ltd. England and Methods in Plant Molecular Biology-A laboratory course manual (1995), Maliga, P., Klessig, D.F., Cashmore, A. R., Gruissem, W. and Varner, J.E. eds. Cold Spring Laboratory Press, New York).

Brassica napus can be transformed as described for example in U.S. PATENT NO. 5 ,188,958 and U.S. PATENT NO. 5 ,463,174. Other *Brassica* such as *rappa, carinata* and *juncea* as well as *Sinapis alba* can be transformed as described by Moloney et. al., (1989, Plant Cell Reports 8: 238-242). Soybean can be transformed by a number of reported procedures. See (U.S. PATENT NO. 5 ,015,580; U.S. PATENT NO. 5 ,015,944; U.S. PATENT NO. 5 ,024,944; U.S. PATENT NO. 5 ,322,783; U.S. PATENT NO. 5 ,416,011; U.S. PATENT NO. 5 ,169,770). A number of transformation procedures have been reported for the production of transgenic maize plants including pollen transformation (U.S. PATENT NO. 5 ,629,183), silicon fiber-mediated transformation (U.S. PATENT NO. 5 ,464,765) electroporation of protoplasts (U.S. PATENT NO. 5 ,231,019; U.S. PATENT NO. 5 ,472,869; U.S. PATENT NO. 5 ,384,253) gene gun (U.S. PATENT NO. 5 ,538,877; U.S. PATENT NO. 5 ,538,880 and *Agrobacterium*-mediated transformation (EP 0 604 662 A1; WO 94/00977). The *Agrobacterium*-mediated procedure is particularly preferred as single integration events of the transgene constructs are more readily obtained

using this procedure which greatly facilitates subsequent plant breeding. Cotton can be transformed by particle bombardment (U.S. PATENT NO. 5 ,004,863; U.S. PATENT NO. 5 ,159,135). Sunflower can be transformed using a combination of particle bombardment and *Agrobacteriuim* infection (EP 0 486 233 A2; U.S. PATENT NO. 5 ,030,572). Flax can be transformed by either particle bombardment or *Agrobacterium*-mediated transformation. Recombinase technologies which are useful in practicing the current invention include the *cre-lox* , FLP/FRT and Gin systems. Methods by which these technologies can be used for the purpose described herein are described, for example, in U.S. PATENT NO. 5 ,527,695; Dale And Ow, 1991, Proc. Natl. Acad. Sci. USA 88: 10558-10562; Sauer, 1993, Methods in Enzymology 225: 890-900; Medberry et. al., 1995, Nucleic Acids Res. 23: 485-490. US 5,723,764 describes a method for controlling plant gene expression using *cre/lox.*

Selectable marker genes include the neomycin phosphotransferase gene *npt*II (U.S. PATENT NO. 5 ,034,322, U.S. PATENT NO. 5 ,530,196), hygromycin resistance gene (U.S. PATENT NO. 5 ,668,298), *bar* gene encoding resistance to phosphinothricin (U.S. PATENT NO. 5 ,276,268). EP 0 530 129 A1 describes a positive selection system which enables the transformed plants to outgrow the non-transformed lines by expressing a transgene encoding an enzyme that activates an inactive compound added to the growth media. Useful screenable marker genes include the ß-glucuronidase gene (Jefferson et. al., 1987, EMBO J. 6: 3901-3907; U.S. PATENT NO. 5 ,268,463) and native or modified green fluorescent protein gene (Cubitt et. al., 1995, Trends Biochem Sci. 20: 448-455; Pang et. al., 1996, Plant Physiol. 112: 893-900). Some of these markers have the added advantage of introducing a trait such as herbicide resistance into the plant of interest providing an additional agronomic value on the input side.

Following transformation by any one of the methods described above, the following procedures can be used to obtain a transformed plant expressing

the transgenes of the current invention: select the plant cells that have been transformed on a selective medium; regenerate the plant cells that have been transformed to produce differentiated plants; and select transformed plants expressing the transgene at such that the level of desired polypeptide is obtained in the desired tissue and cellular location.

The examples demonstrate the synthesis of new genetically engineered enzymes for the efficient production of polyhydroxyalkanoate biopolymers in transgenic organisms. In one example, the thiolase and reductase activities encoded by the *phbA* and *phbB* genes have been combined into a single enzyme through the construction of a gene fusion. Use of such a hybrid enzyme and its corresponding gene is advantageous: combining two enzyme activities in a single transcriptional unit reduces the number of genes that need to be expressed in transgenic organisms, and the close proximity of two enzyme activities which catalyse sequential steps in a metabolic pathway. On the fusion enzyme allows for direct transfer of the reaction product from the first catalytic domain to the second domain. These gene fusions can be applied in transgenic microbial or plant crop PHA production systems. The fusions can be expressed in the cytosol or subcellular organelles of higher plants such as the seed of an oil crop (*Brassica*, sunflower, soybean, corn, safflower, flax, palm or coconut), starch accumulating plants (potato, tapioca, cassava), fiber plants (cotton, hemp) or the green tissue of tobacco, alfalfa, switchgrass or other forage crops.

Examples

The present invention will be further understood by reference to the following examples, which use these general methods and materials:

DNA manipulations were performed on plasmid and chromosomal DNA purified with the Qiagen plasmid preparation or Qiagen chromosomal DNA preparation kits according to manufacturers recommendations. DNA was digested using restriction enzymes (New England Biolabs, Beverly, MA) according to manufacturers recommendations. DNA fragments were isolated from 0.7% agarose-Tris/acetate/EDTA gels using a Qiagen kit.

Oligonucleotides were purchased from Biosynthesis or Genesys. DNA sequences were determined by automated sequencing using a Perkin-Elmer ABI 373A sequencing machine. DNA was amplified using the polymerase-chain-reaction in 50 microliter volume using PCR-mix from Gibco-BRL (Gaithersburg, Md) and an Ericomp DNA amplifying machine.

E. coli strains were grown in Luria-Bertani medium or 2xYT medium (Sambrook et. al., 1992, in Molecular Cloning, a laboratory manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY). at 37 °C, 30 °C or 16 °C.

Accumulated PHB was determined by gas chromatographic (GC) analysis, carried out on the lyophilized cell mass. About 20 mg of lyophilized cell mass was subjected to simultaneous extraction and butanolysis at 110°C for 3 hours in 2 mL of a mixture containing (by volume) 90% 1-butanol and 10% concentrated hydrochloric acid, with 2 mg/mL benzoic acid added as an internal standard. The water-soluble components of the resulting mixture were removed by extraction with 3 mL water. The organic phase (1 µL at a split ratio of 1:50 at an overall flow rate of 2 mL/min) was analyzed on an HP 5890 GC with FID detector (Hewlett-Packard Co, Palo Alto, CA) using an SPB-1 fused silica capillary GC column (30 m; 0.32 mm ID; 0.25 µm film; Supelco; Bellefonte, Pa.) with the following temperature profile: 80 °C, 2 min; 10 C° per min to 250 °C; 250 °C, 2 min. Butylbenzoate was used as an internal standard. Molecular weights of the isolated polymers were determined by GPC using a Waters Styragel HT6E column (Millipore Corp., Waters Chromatography Division, Milford, MA) calibrated vs. polystyrene samples of narrow polydispersity. Samples were dissolved in chloroform at 1 mg/mL, 50 µL samples were injected and eluted at 1 mL/min. Detection was performed using a differential refractometer.

Protein samples were denatured by incubation in a boiling water bath (3 minutes) in the presence of 2-mercaptoethanol and sodium dodecylsulphate and subsequently separated on 10%, 15% or 10-20% sodium dodecylsulphate-

polyacrylamide gels (SDS-PAGE). After transfer of protein to supported nitrocellulose membranes (Gibco-BRL, Gaithersburg, MD), 3-ketoacyl-CoA thiolase, acetoacetyl-CoA reductase and PHB polymerase were detected using polyclonal antibodies raised against these enzymes in rabbits and horse-radish peroxidase labeled secondary antibodies followed by chemiluminescent detection (USB/Amersham).

ß-ketothiolase and NADP-specific acetoacetyl-CoA reductase activities were measured as described by Nishimura et al. (1978, Arch. Microbiol. 116: 21-24) and Saito et al. (1977, Arch. Microbiol. 114: 211-217) respectively. The acetoacetyl-CoA thiolase activity is measured as degradation of a Mg^{2+}-acetoacetyl-CoA complex by monitoring the decrease in absorbance at 304 nm after addition of cell free extract using a Hewlett-Packer spectrophotometer. The acetoacetyl-CoA reductase activity is measured by monitoring the conversion of NADPH to NADP at 340 nm using a Hewlett-Packer spectrophotometer.

Example 1: Construction of thiolase-reductase fusion protein (Thredase)

Plasmid pTrc AB11 was constructed using the following techniuqes essentially as illustrated in Figure 2. The *phbA* gene from *A. eutrophus* was amplified from plasmid pAeT413, a derivative of plasmid pAeT41 (Peoples, O.P. and Sinskey, A.J., 1989, J. Biol. Chem. 264: 15298-15303): by thermal cycling (30 cycles of 40 sec. at 94 °C, 40 sec. at 65 °C and 2 min at 72 °C, followed by a final extension step at 72 °C for 7 min.) with the following primers. The DNA sequence and the amino acid sequence of *phbA* from *A. eutrophus* is shown in SEQ ID NO: 1 and SEQ ID NO: 2

A1FKpn
(GGGGTACCAGGAGGTTTTTATGACTGACGTTGTCATCGTATCC)
(SEQ ID NO: 3)

A1F-Bam
(CGCGGATCCTTTGCGCT CGACTGCCAGCGCCACGCCC).
(SEQ ID NO: 4)

A1F-Kpn contains the ribosome binding site and translational start site; A1F-Bam does not include the translational stop codon. The *A. eutrophus phb*B gene was amplified from a derivative of plasmid pAeT41 (Peoples, O.P. and Sinskey, A.J., 1989, J. Biol. Chem. 264: 15298-15303) by thermal cycling (30 cycles of 40 sec. at 94 °C, 40 sec. at 45 °C and 2 min at 72 °C, followed by a final extension step at 72 °C for 7 min.) with the following primers. The DNA sequence and the amino acid sequence of *phbB* from *A. eutrophus* is shown in SEQ ID NO: 5 and SEQ ID NO: 6.

B1L-Bam
(CGCGGATCCATGACTCAG CGCATTGCGTATGT GACC)
(SEQ ID NO: 7)

B1L-Xba
(GCTCTAGATCAGCCCATATGCAGGC CGCCGTTGAGCG).
(SEQ ID NO: 8)

B1L-Bam contains an ATG initiation codon next to the *BamHI* site but no translational intiation signals; B1L-Xba contains the translational stop codon TGA. The amplified *phbA* gene was then digested with *KpnI* and *BamHI*, and the amplified *phbB* gene was digested with *BamHI* and *XbaI*. Following digestion, the *phb*A gene was cloned into pTrcN which had been digested with *KpnI* and *BamHI* to produce pTrcAF and the *phb*B gene was cloned into *BamHI/XbaI*-digested pTrcN to produce pTrcBL.

After confirmation of the DNA sequence of the insert, *phb*B was cloned as a *BamHI/XbaI* fragment from pTrcBL into *BamHI/XbaI* digested pTrcAF

resulting in plasmid pTrcAB11. The resulting hybrid gene encodes for a thiolase-glycine-serine-reductase fusion. The DNA sequence and the amino acid sequence of the AB11 fusion is shown in SEQ ID NO: 9 and SEQ ID NO: 10.

The insertion of the *BamHI* site between *phbA* and *phbB* results in a glycine-serine linker that connects the thiolase and the reductase enzyme and which could be subsequently modified to alter the length and/or sequence of the linker region. Several such derivatives of pTrcAB11 were constructed as follows: pTrcAB11 was digested with *BamHI* and the linearized fragment purified and dephosphorylated with shrimp alkaline phosphatase.

Oligonucleotides were designed to insert the following DNA fragments into the *BamHI* site. The encoded amino acid sequence is indicated:

L5A	5' GATCTACCG 3'	(SEQ ID NO: 11)
L5B	3' ATGGCCTAG 5'	(SEQ ID NO: 12)
	G S T G S	(SEQ ID NO: 13)

Oligonucleotides L5A and L5B (500 pmol) were phosphorylated using T4 polynucleotide kinase and annealed (133 pmol of each primer) and ligated into linearized pTrcAB11. The ligation mixture was electroporated into *E. coli* MBX240 and plasmids with the linker inserted between the thiolase and reductase genes were identified by restriction enzyme digestion with BsaWI.

The utility of the fusion constructs was investigated by transforming them into *E. coli* MBX240 and examining the integrity of the fusion at the polypeptide level by immunoblotting at the protein level by enzyme assays and for the production of PHB. MBX240 was derived from *E. coli* XL1-blue by integration of the *A. eutrophus phaC* gene (Peoples, O.P. and Sinskey, A.J., 1989, J. Biol. Chem. 264: 15298-15303). An alternative approach to the integrated strain would be to have expressed the PHB synthase from a compatible plasmid.

Recombinant strains containing the appropriate fusion plasmid were grown overnight in 2xYT/1% glucose/100 μg/ml ampicillin at 30 C. The grown culture was diluted 1:100 into 50 ml of fresh 2xYT/1% glucose/100 μg/ml ampicillin and incubated at 30 C. Two identical sets of cultures were inoculated, one which was induced with IPTG and one was not induced. Once the culture reached an OD_{600} of 0.6, samples were induced with a final concentration of 1 mM IPTG. Cells were harvested 24 hours after induction by splitting into two 50ml samples and centrifugation at 3000 x g for 10 minutes. Samples of whole cells were retained for analysis of PHB content. The second set of pellets were resuspended in 0.75 ml of lysis buffer (50 mM Tris, 1 mM EDTA, 20% glycerol, pH 8.2) and sonicated (50% output, 2 min. at 50%). The crude extract was then centrifuged (10 min 3000xg, 4 °C) and the supernatant and pellet were separated on 10% SDS-PAGE gels and analyzed by Coomassie staining as well as by immuno-blotting. Immuno-blots were probed with rabbit anti-*A.* eutrophus thiolase and rabbit anti-*A. eutrophus* reductase antibodies. Both antibodies reacted with an Mr = 62kD protein which was absent from the control strain, MBX240 containing the vector pTrcN alone. There was no cross reactivity of the anti-thiolase antibodies with an Mr 42 kD polypeptide or of the reductase antibodies with an Mr 26 kD polypeptide. The soluble protein was then analyzed for thiolase and reductase activity.

The results of these analysis are presented in Table 1 for pTrcAB11 and five derivatives with modified linkers.

Table 1: Fusion Enzyme Activities

fusion[a]	induction[b]	thiolase activity[c]	reductase activity[c]	%PHB[d]
pTrcN	-	0.03	0.05	0
	+	0.03	0.03	0
AB11	-	0.15	0.09	28.6
	+	0.32	0.07	56.3
L5-1	-	0.44	0.08	32.4
	+	0.97	0.12	62.5
L5-2	-	0.25	0.07	34.2
	+	0.37	0.09	57.6
L5-3	-	0.38	0.06	40.4
	+	1.18	0.09	63.6
L5-4	-	0.51	0.11	37.6
	+	2.21	0.17	65.3
L5-5	-	0.44	0.11	36.0
	+	1.85	0.23	64.1

[a] construct inserted in pTrcN, L5-n indicates an AB11 fusion with a linker derived from the L5 oligonucleotide set; [b] culture was induced (+) 1 mM IPTG at an OD600 for 24 hours or was uninduced (-); [c] thiolase and reductase activity in U/mg of crude protein extract; [d] accumulated PHB as percentage of the cell dry weight.

The results presented in Table 1 indicate that these thiolase-reductase fusions have both enzyme activities and result in the production of high levels of PHB.

The fusion encoded by pTrcAB11 was partially purified. A culture of *E. coli* MBX240 (XL1-Blue::*phbC150*) [pTrcAB11] cells grown at 16 °C for 33 hours (5.5 g) were resuspended in 11 ml of lysis buffer (50 mM Tris, 1 mM EDTA, 0.05% (w/v) Hecameg, 20% glycerol, pH 8.0) and sonicated (50% output, 2 min at 50%). The crude extract was then centrifuged (10 min 3000xg, 4 °C) and the supernatant was applied to a pre-equilibrated Toyopearl DEAE 650S (Rohm & Haas, PA) column (16.5 x 3.0 cm) in 50 mM NaCl. Unbound protein was washed off with a 50 mM NaCl (300 ml) after which bound protein was eluted with a 50-500 mM NaCl gradient (400 ml total volume). Fractions containing both thiolase and reductase activity (eluted at 250 mM NaCl) were

pooled and concentrated/desalted on a 50,000 MW spin column (Amicon). The active protein sample was further purified over a BLUE-SEPHAROSE™ CL6B (Pharmacia Biotech AB, Sweden) column (10.5 cm x 2.6 cm) using the same buffers as for the DEAE but containing different NaCl concentrations. Unbound protein was washed off the column with 250 mM NaCl (200ml) and the remaining protein was eluted in two steps using 750 mM NaCl and 2M NaCl. Two thirds of the thiolase and reductase activities were recovered in the 750 mM NaCl step with the remainder eluting in the 2M NaCl step. Again, fractions containing both thiolase and reductase activity were pooled and concentrated/ desalted on a 50,000 MW spin column . The fusion protein preparation was analyzed by SDS-PAGE proteins detected by either Coomassie Blue staining or Western-blot analysis using anti-ß-ketothiolase and anti-acetoacetyl-CoA reductase antibodies. Fractions that contained both ß-ketothiolase and acetoacetyl-CoA reductase activity showed a single protein band with an apparent molecular weight of 60 kDa that reacted with both antibodies, confirming both enzyme activities were present on a single polypeptide chain encoded by a single gene.

Example 2: Construction of reductase-thiolase fusion protein

A hybrid gene that expresses a reductase-glycine-serine-thiolase enzyme was constructed from PCR products containing the reductase and thiolase genes. The following primers

B1F-Kpn
(GGGGTACCAGGAGGTTTTTATGACTCAGCGCATTGCGTATGTGACC) (SEQ ID NO: 14)

B1F-BamHI
(CGCGGATCCGCCCATATGCAGGCCGCCGTTGAGCG) (SEQ ID NO: 15)

A1L-BamHI
(CGCGGATCCATGACTGACGTTGTCATCGTATCC) (SEQ ID NO: 16)

A1L-XbaI
(GCTCTAGATTATTTGCGCTCGACTGCCAGCGCCACGCCC)
(SEQ ID NO: 17)

were used to amplify (30 cycles of 40 sec. at 94 °C, 40 sec. at 65 °C and 2 min at 72 °C, followed by a final extension step at 72°C for 7 min.) these genes such that the reductase gene is preceded by a ribosome binding site and does not contain a stop codon. The stop codon of the fusion is provided by the thiolase gene.

The amplified *phbB* gene was digested with *KpnI* and *BamHI*, then cloned into the *KpnI-BamHI* site of pTrcN to produce pTrcBF. The amplified *phbA* gene was digested with *BamHI* and *XbaI*, and was cloned into the *BamHI-XbaI* site of pTrcN to obtain plasmid pTrcAL. The *phb*B gene from pTrcBF was digested with *BamHI-KpnI* and the fragment was inserted it into the *BamHI-KpnI* site of pTrcAL to obtain plasmid pTrcBA, resulting in a fusion gene coding for reductase-glycine-serine-thiolase in one polypeptide. The DNA sequence and the amino acid sequence of the B1A1 fusion is shown in SEQ ID NO: 18 and SEQ ID NO: 19.

Example 3: Design of PHA synthase-ACP::CoA transferase fusions

The *phaC1* gene encoding PHA synthase 1 of *P. oleovorans* (Huisman et. al., 1991, J. Biol. Chem. 266: 2191-2198) (C3) can be amplified by polymerase chain reaction using the following primers. The DNA sequence and the amino acid sequence of *phbC1* gene of *P. oleovorans* is shown in SEQ ID NO: 20 and SEQ ID NO: 21.

C3 up I

5' g-GAATTC-aggaggtttt-ATGAGTAACAAGAACAACGATGAGC 3'
(SEQ ID NO: 22)

C3 up II
5' CG-GGATCC-acgctcgtgaacgtaggtgccc 3' (SEQ ID NO: 23)

C3 dw I
5' CG-GGATCC-AGTAACAAGAACAACGATGAGC 3'
(SEQ ID NO: 24)

C3 dw II
5' GC-TCTAGA-AGCTT-TCAACGCTCGTGAACGTAGGTGCCC 3'
(SEQ ID NO: 25)

.

The *pha*G gene encoding acyl-ACP::CoA transferase from *P. putida* (G3) can be amplified by polymerase chain reaction using the following primers. The DNA sequence and the amino acid sequence of *phaG* gene of *P. putida* are shown in SEQ ID NO: 26 and SEQ ID NO: 27.

G3 dw I
5' CG-GGATCC-AGGCCAGAAATCGCTGTACTTG 3' (SEQ ID NO: 28)

G3 dw II
5' GC-TCTAGA-AGCTT-TCAGATGGCAAATGCATGCTGCCCC 3'
(SEQ ID NO: 29)

G3 up I
5' G-GAATTC-AGGAGGTTTT-ATGAGGCCAGAAATCGCTGTACTTG 3'
(SEQ ID NO: 30)

G3 up II

5' CG-GGATCC-GATGGCAAATGCATGCTGCCCC 3'.

(SEQ ID NO: 31)

Fusions of C3 and G3 are subsequently created by cloning either the C3 up and G3 dw PCR products, or the G3 up and C3 dw PCR products as *EcoRI-BamHI* and *BamHI-HindIII* fragments into pTrcN. The resulting plasmids code for either a synthase-transferase fusion (C3G3) or transferase-synthase (G3C3) fusion protein. The DNA sequence and the amino acid sequence of C3G3 is shown in SEQ ID NO: 32 and SEQ ID NO: 33, and the DNA sequence and the amino acid sequence of G3C3 gene are shown in SEQ ID NO: 34 and SEQ ID NO: 35.

Example 4: Design of PHA synthase-hydratase fusions

The *phaC* gene encoding a PHB synthase fusion from *Z. ramigera* (C5) was amplified by polymerase chain reaction using the following primers. The DNA sequence and the amino acid sequence of *phbC* gene of *Z. ramigera* are shown in SEQ ID NO: 36 and SEQ ID NO: 37.

C5 up I

5' G-GAGCTC-AGGAGGTTTT-ATGAGTAACAAGAACAACGATGAGC 3'

(SEQ ID NO: 38)

C5 up II

5' CG-GGATCC-GCCCTTGGCTTTGACGTAACGG 3' (SEQ ID NO: 39)

C5 dw I

5' CG-GGATCC-AGTAACAAGAACAACGATGAGC 3' (SEQ ID NO: 40)

C5 dw II

5' GC-TCTAGA-AGCTT-TCAGCCCTTGGCTTTGACGTAACGG 3'

(SEQ ID NO: 41)

The phaJ gene encoding (*R*)-specific enoyl-CoA transferase from *A. caviae* (J12) can be amplified by polymerase chain reaction using the following primers. The DNA sequence and the amino acid sequence of *phbJ* gene of *A. caviae* are shown in SEQ ID NO: 42 and SEQ ID NO: 43.

J12 dw I
5' CG-GGATCC-AGCGCACAATCCCTGGAAGTAG 3'
(SEQ ID NO: 44)

J12 dw II
5' GC-TCTAGA-AGCTT-TTAAGGCAGCTTGACCACGGCTTCC 3'
(SEQ ID NO: 45)

J12 up I
5' AG-GAGCTC-AGGAGGTTTT-ATGAGCGCACAATCCCTGGAAGTAG 3'
(SEQ ID NO: 46)

J12 up II
5' CG-GGATCC-AGGCAGCTTGACCACGGCTTCC 3' (SEQ ID NO: 47)

Fusions of C5 and J12 are subsequently created by cloning either the C5 up and J12 dw PCR products, or the J12 up and C5 dw PCR products as *EcoRI-BamHI* and *BamHI-HindIII* fragments into pTrcN. The resulting plasmids encode either a synthase-hydratase (C5J12) or hydratase-synthase (J12C5) fusion enzyme. The DNA sequence and the amino acid sequence of C5J12 RE shown in SEQ ID NO: 48 and SEQ ID NO: 49, and the DNA sequence and the amino acid sequence of J12C5 gene are shown in SEQ ID NO: 50 and SEQ ID NO: 51.

Example 5: Design of broad-substrate range thiolase-reductase fusions

The *bktB* gene encoding thiolase II of *R. eutropha* (Slater et al. J. Bacteriol. (1998) 180, 1979-1987) (A1-II) can be amplified by polymerase chain reaction using the following primers. The DNA sequence and the amino acid sequence of *bktB* gene of *R. eutropha* are shown in SEQ ID NO: 52 and SEQ ID NO: 53.

A1-II up I

5' G-GAATTC-AGGAGGTTTT-ATGACGCGTGAAGTGGTAGTGGTAAG 3' (SEQ ID NO: 54)

A1-II up II

5' CG-GGATCC-GATACGCTCGAAGATGGCGGC 3' (SEQ ID NO: 55)

A1-II dw I

5' CG-GGATCC-ACGCGTGAAGTGGTAGTGGTAAG 3' (SEQ ID NO: 56)

A1-II dw II

5' GC-TCTAGA-AGCTT-TCAGATACGCTCGAAGATGGCGGC 3' (SEQ ID NO: 57)

The *phaB* gene encoding acyl-CoA reductase from *R. eutropha* (B1) is amplified by polymerase chain reaction using the primers described in Example 1. Fusions of A1-II and B1 are subsequently created by cloning either the A1-II up and B1 dw PCR products, or the B1 up and A1-II dw PCR products as *EcoRI-BamHI* and *BamHI-HindIII* fragments into pTrcN. The resulting plasmids encode either a thiolase-reductase (A1-IIB1) or reductase-thiolase (B1A1-II)) fusion enzyme. The DNA sequence and the amino acid sequence of A1-IIB1is shown in SEQ ID NO: 58 and SEQ ID NO: 59, and the DNA

sequence and the amino acid sequence of B1A1-II gene are shown in SEQ ID NO: 60 and SEQ ID NO: 61.

Modifications and variations of the present invention will be obvious to those of skill in the art from the foregoing detailed description. Such modifications and variations are intended to come within the scope of the following claims.

We claim:

1. Protein fusions having a formula selected from the group consisting of E1-L_n-E2 or E2-L_n-E1, wherein E1 and E2 are selected from the group comprising ß-ketothiolases, acyl-CoA reductases, PHA synthases, PHB synthetases, phasins, enoyl-CoA hydratases and beta-hydroxyacyl-ACP::coenzyme-A transferase, in which L_n is a peptide of n amino acids that links E1 to E2 or E2 to E1.

2. The fusion of claim 1 selected from the group consisting of beta-ketothiolase (phbA) and acyl-CoA reductase (phbB); phbB and phbA ; PHA synthase (phaC) and phasin (phaP); phaP and phaC (1D); phaC and beta-hydroxyacyl-ACP::coenzyme-A transferase (phbG); phbG and phaC; phaC and enoyl-CoA hydratases (phaJ); and phaJ and phaC.

3. The fusion of claim 1 wherein n in the linker is between zero and 50 amino acids.

4. The fusion of claim 1 wherein the linker is glycine-serine.

5. The fusion of claim 1 expressed in a plant.

6. The fusion of claim 1 expressed in a bacteria.

7. A gene encoding protein fusions having a formula selected from the group consisting of E1-L_n-E2 or E2-L_n-E1, wherein E1 and E2 are selected from the group comprising ß-ketothiolases, acyl-CoA reductases, PHA synthases, PHB synthetases, phasins, enoyl-CoA hydratases and beta-hydroxyacyl-ACP::coenzyme-A transferase, in which L_n is a peptide of n amino acids that links E1 to E2 or E2 to E1.

8. The gene of claim 7 encoding a fusion protein selected from the group consisting of beta-ketothiolase (phbA) and acyl-CoA reductase (phbB); phbB and phbA ; PHA synthase (phaC) and phasin (phaP); phaP and phaC (1D); phaC and beta-hydroxyacyl-ACP::coenzyme-A transferase (phbG); phbG and phaC; phaC and enoyl-CoA hydratases (phaJ); and phaJ and phaC.

9. The gene of claim 7 wherein n in the linker is between zero and 50 amino acids.

10. The gene of claim 7 wherein the linker is glycine-serine.

11. The gene of claim 7 comprising a promoter for expression in plants.

12. The gene of claim 11 comprising a promoter specific for expression in a tissue, plastid or other organ.

13. The gene of claim 11 comprising a promoter specific for expression during a regulatory phase.

14. The gene of claim 7 further comprising RNA processing signals or ribozyme sequences.

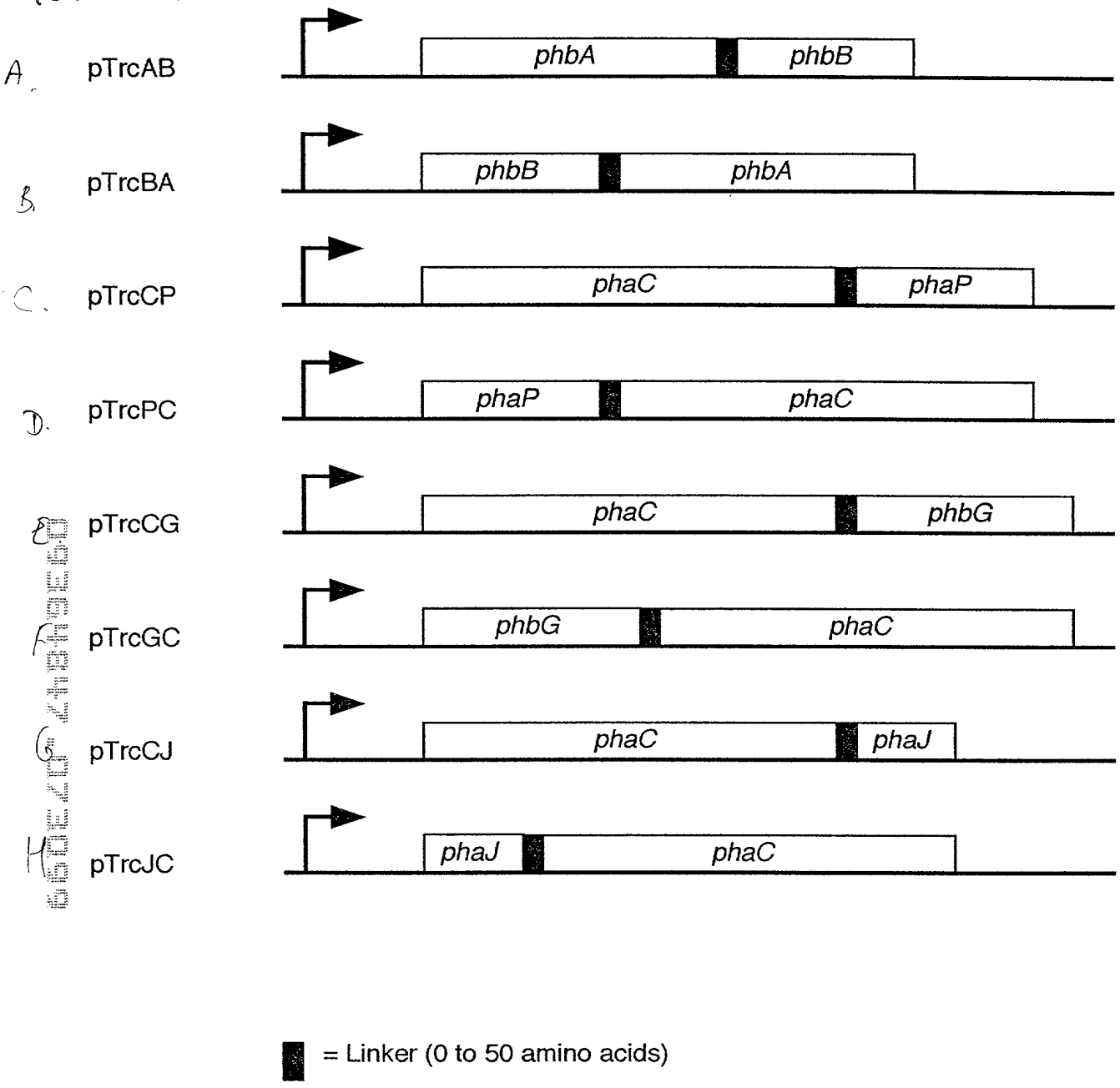
ENZYMES FOR BIOPOLYMER PRODUCTION

Abstract of the Disclosure

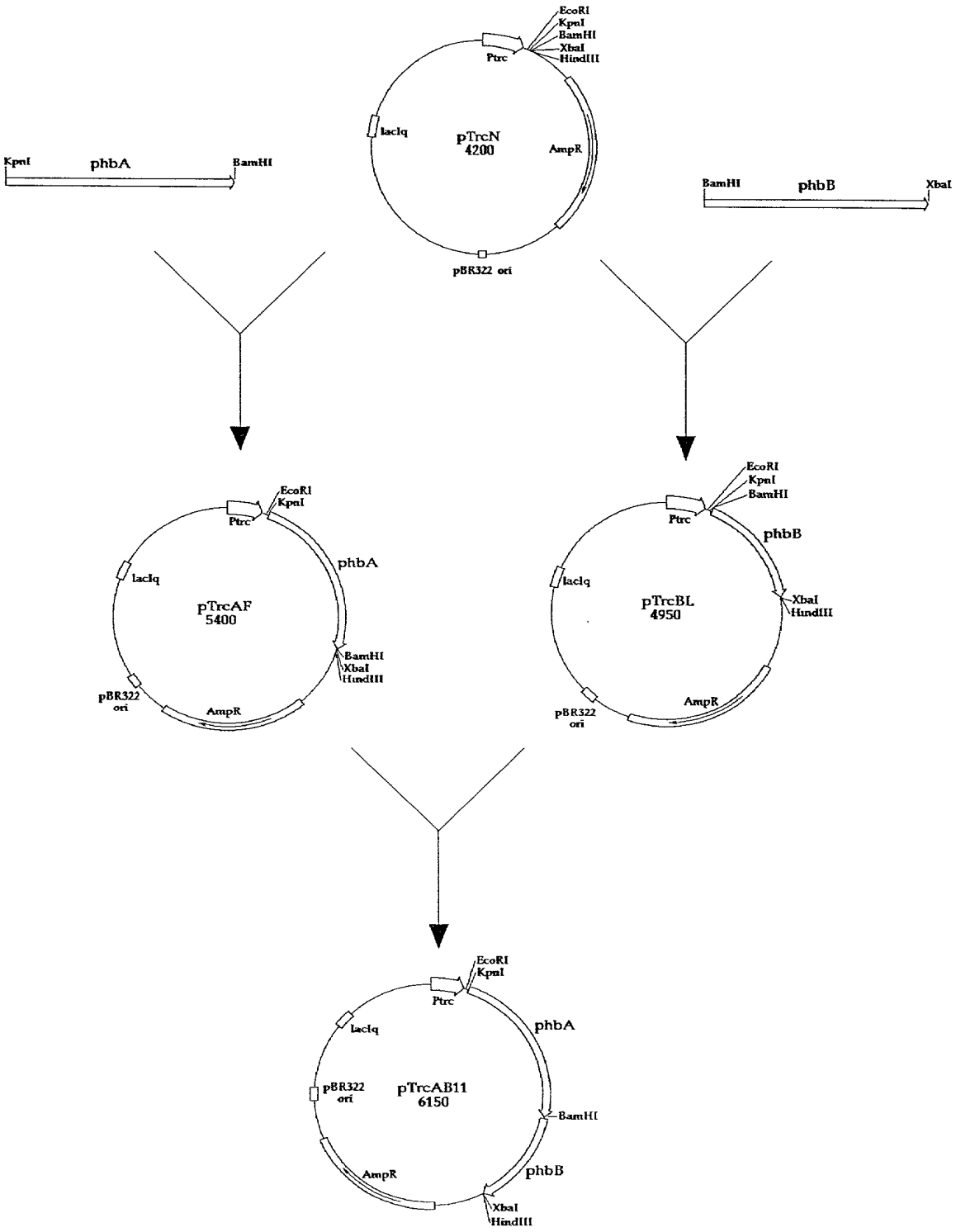
In order to optimize the flux or flow of carbon intermediates from normal cellular metabolism into PHAs it is desirable to optimize the **expression of the** enzymes of the PHA biosynthetic pathway. Gene fusions are genetic constructs where two open reading frames have been fused into one and encode hybrid proteins and in some cases bifunctional hybrid enzymes. Linkers may be added to spatially separate the two domains of the hybrid protein. In the case of enzymes which catalyse successive reactions in a pathway, the fusion of two genes results in bringing two enzymatic activities into close proximity to each other. When the product of the first reaction is a substrate for the second one, this new configuration of active sites may result in a faster transfer of the product of the first reaction to the second active site with a potential for increasing the flux through the pathway.

MBX-30 FIGURE 1

FIGURES 1A-1H



MBX-0 0 FIGURE 2



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DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)

☒ Declaration Submitted with Initial Filing **OR** ☐ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)

Attorney Docket Number	MBX 030
First Named Inventor	Oliver P. Peoples
COMPLETE IF KNOWN	
Application Number	/
Filing Date	July 30, 1999
Group Art Unit	
Examiner Name	

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

ENZYMES FOR BIOPOLYMER PRODUCTION

(Title of the Invention)

the specification of which
☒ is attached hereto
OR
☐ was filed on (MM/DD/YYYY) _____ as United States Application Number or PCT International Application Number _____ and was amended on (MM/DD/YYYY) _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached? YES	NO
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)
60/094,674	July 30, 1998

☐ Additional provisional application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

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I hereby claim the benefit under 35 U.S.C. 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number *(if applicable)*

☐ Additional U.S. or PCT international application numbers are lis'ed on a supplemental priority data sheet PTO/SB/02B attached hereto.

As a named inventor, I hereby appoint the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: ☐ Customer Number [ ] → *Place Customer Number Bar Code Label here*

OR

☒ Registered practitioner(s) name/registration number listed below

Name	Registration Number	Name	Registration Number
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Robert A. Hodges	41,074		
Kevin W. King	42,737		

☐ Additional registered practitioner(s) named on supplemental Registered Practitioner Information sheet PTO/SB/02C attached hereto.

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])	Family Name or Surname
Oliver P.	Peoples

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City	Arlington	State	MA	ZIP	02174	Country	US

☐ Additional inventors are being named on the ____supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto

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DECLARATION

ADDITIONAL INVENTOR(S)
Supplemental Sheet
Page ___ of ___

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor
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Inventor's Signature						**Date**	
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Post Office Address							
City	Brookline	**State**	MA	**ZIP**	02142	**Country**	US

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor
Given Name (first and middle [if any])	Family Name or Surname
Gjalt	Huisman

Inventor's Signature						**Date**	
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Post Office Address	3370 Brittan Avenue, #18						
Post Office Address							
City	San Carlos	**State**	CA	**ZIP**	94070	**Country**	US

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor
Given Name (first and middle [if any])	Family Name or Surname

Inventor's Signature						**Date**	
Residence: City		**State**		**Country**		**Citizenship**	
Post Office Address							
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gatgcggtga tcgacaccaa cctgacctcg ctgttcaacg tcaccaagca ggtgatcgac 1560
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atcccggtca agcgcctggg cctgccggaa gagatcgcct cgatctgcgc ctggttgtcg 1860
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ggctga 1926

<210> 10
<211> 641
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Thredase
Fusion Protein

<220>
<221> PEPTIDE
<222> (1)..(641)

<400> 10
Met Thr Asp Val Val Ile Val Ser Ala Ala Arg Thr Ala Val Gly Lys
1 5 10 15

Phe Gly Gly Ser Leu Ala Lys Ile Pro Ala Pro Glu Leu Gly Ala Val
20 25 30

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Val Ile Lys Ala Ala Leu Glu Arg Ala Gly Val Lys Pro Glu Gln Val
        35                  40                  45

Ser Glu Val Ile Met Gly Gln Val Leu Thr Ala Gly Ser Gly Gln Asn
    50                  55                  60

Pro Ala Arg Gln Ala Ala Ile Lys Ala Gly Leu Pro Ala Met Val Pro
65                  70                  75                  80

Ala Met Thr Ile Asn Lys Val Cys Gly Ser Gly Leu Lys Ala Val Met
                85                  90                  95

Leu Ala Ala Asn Ala Ile Met Ala Gly Asp Ala Glu Ile Val Val Ala
            100                 105                 110

Gly Gly Gln Glu Asn Met Ser Ala Ala Pro His Val Leu Pro Gly Ser
        115                 120                 125

Arg Asp Gly Phe Arg Met Gly Asp Ala Lys Leu Val Asp Thr Met Ile
    130                 135                 140

Val Asp Gly Leu Trp Asp Val Tyr Asn Gln Tyr His Met Gly Ile Thr
145                 150                 155                 160

Ala Glu Asn Val Ala Lys Glu Tyr Gly Ile Thr Arg Glu Ala Gln Asp
                165                 170                 175

Glu Phe Ala Val Gly Ser Gln Asn Lys Ala Glu Ala Ala Gln Lys Ala
            180                 185                 190

Gly Lys Phe Asp Glu Glu Ile Val Pro Val Leu Ile Pro Gln Arg Lys
        195                 200                 205

Gly Asp Pro Val Ala Phe Lys Thr Asp Glu Phe Val Arg Gln Gly Ala
    210                 215                 220

Thr Leu Asp Ser Met Ser Gly Leu Lys Pro Ala Phe Asp Lys Ala Gly
225                 230                 235                 240

Thr Val Thr Ala Ala Asn Ala Ser Gly Leu Asn Asp Gly Ala Ala Ala
                245                 250                 255

Val Val Val Met Ser Ala Ala Lys Ala Lys Glu Leu Gly Leu Thr Pro
            260                 265                 270

Leu Ala Thr Ile Lys Ser Tyr Ala Asn Ala Gly Val Asp Pro Lys Val
        275                 280                 285
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Met Gly Met Gly Pro Val Pro Ala Ser Lys Arg Ala Leu Ser Arg Ala
290 295 300

Glu Trp Thr Pro Gln Asp Leu Asp Leu Met Glu Ile Asn Glu Ala Phe
305 310 315 320

Ala Ala Gln Ala Leu Ala Val His Gln Gln Met Gly Trp Asp Thr Ser
325 330 335

Lys Val Asn Val Asn Gly Gly Ala Ile Ala Ile Gly His Pro Ile Gly
340 345 350

Ala Ser Gly Cys Arg Ile Leu Val Thr Leu Leu His Glu Met Lys Arg
355 360 365

Arg Asp Ala Lys Lys Gly Leu Ala Ser Leu Cys Ile Gly Gly Gly Met
370 375 380

Gly Val Ala Leu Ala Val Glu Arg Lys Gly Ser Met Thr Gln Arg Ile
385 390 395 400

Ala Tyr Val Thr Gly Gly Met Gly Gly Ile Gly Thr Ala Ile Cys Gln
405 410 415

Arg Leu Ala Lys Asp Gly Phe Arg Val Val Ala Gly Cys Gly Pro Asn
420 425 430

Ser Pro Arg Arg Glu Lys Trp Leu Glu Gln Gln Lys Ala Leu Gly Phe
435 440 445

Asp Phe Ile Ala Ser Glu Gly Asn Val Ala Asp Trp Asp Ser Thr Lys
450 455 460

Thr Ala Phe Asp Lys Val Lys Ser Glu Val Gly Glu Val Asp Val Leu
465 470 475 480

Ile Asn Asn Ala Gly Ile Thr Arg Asp Val Val Phe Arg Lys Met Thr
485 490 495

Arg Ala Asp Trp Asp Ala Val Ile Asp Thr Asn Leu Thr Ser Leu Phe
500 505 510

Asn Val Thr Lys Gln Val Ile Asp Gly Met Ala Asp Arg Gly Trp Gly
515 520 525

Arg Ile Val Asn Ile Ser Ser Val Asn Gly Gln Lys Gly Gln Phe Gly
530 535 540

Gln Thr Asn Tyr Ser Thr Ala Lys Ala Gly Leu His Gly Phe Thr Met
545 550 555 560

Ala Leu Ala Gln Glu Val Ala Thr Lys Gly Val Thr Val Asn Thr Val
565 570 575

Ser Pro Gly Tyr Ile Ala Thr Asp Met Val Lys Ala Ile Arg Gln Asp
580 585 590

Val Leu Asp Lys Ile Val Ala Thr Ile Pro Val Lys Arg Leu Gly Leu
595 600 605

Pro Glu Glu Ile Ala Ser Ile Cys Ala Trp Leu Ser Ser Glu Glu Ser
610 615 620

Gly Phe Ser Thr Gly Ala Asp Phe Ser Leu Asn Gly Gly Leu His Met
625 630 635 640

Gly

<210> 11
<211> 9
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide primer- L5A

<400> 11
gatctaccg 9

<210> 12
<211> 9
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide primer- L5B

<400> 12
atggcctag 9

<210> 13

<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Peptide Linker

<220>
<221> PEPTIDE
<222> (1)..(5)

<400> 13
Gly Ser Thr Gly Ser
1 5

<210> 14
<211> 46
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide primer- B1F-Kpn

<400> 14
ggggtaccag gaggttttta tgactcagcg cattgcgtat gtgacc 46

<210> 15
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide primer- B1F-BamHI

<400> 15
cgcggatccg cccatatgca ggccgccgtt gagcg 35

<210> 16
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide primer- A1L BamHI

<400> 16
cgcggatcca tgactgacgt tgtcatcgta tcc 33

<210> 17
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide primer- A1L-XbaI

<400> 17
gctctagatt atttgcgctc gactgccagc gccacgccc 39

<210> 18
<211> 1926
<212> DNA
<213> Artificial Sequence

<220>
<221> gene
<222> (1)..(1926)
<223> phbB-linker-phbA fusion gene

<400> 18
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cgcgaaaagt ggctggagca gcagaaggcc ctgggcttcg atttcattgc ctcggaaggc 180
aatgtggctg actgggactc gaccaagacc gcattcgaca aggtcaagtc cgaggtcggc 240
gaggttgatg tgctgatcaa caacgccggt atcacccgcg acgtggtgtt ccgcaagatg 300
acccgcgccg actgggatgc ggtgatcgac accaacctga cctcgctgtt caacgtcacc 360
aagcaggtga tcgacggcat ggccgaccgt ggctggggcc gcatcgtcaa catctcgtcg 420
gtgaacgggc agaagggcca gttcggccag accaactact ccaccgccaa ggccggcctg 480
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gtctctccgg gctatatcgc caccgacatg gtcaaggcga tccgccagga cgtgctcgac 600
aagatcgtcg cgacgatccc ggtcaagcgc ctgggcctgc cggaagagat cgcctcgatc 660
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gaggcgcagg atgagttcgc cgtcggctcg cagaacaagg ccgaagccgc gcagaaggcc 1320
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aagggcctgg cctcgctgtg catcggcggc ggcatgggcg tggcgctggc agtcgagcgc 1920
aaataa 1926

<210> 19
<211> 641
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Reductase
Fusion Protein

<220>
<221> PEPTIDE
<222> (1)..(641)

<400> 19

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Thr	Ala	Ile	Cys	Gln	Arg	Leu	Ala	Lys	Asp	Gly	Phe	Arg	Val	Val	Ala
		20					25					30			
Gly	Cys	Gly	Pro	Asn	Ser	Pro	Arg	Arg	Glu	Lys	Trp	Leu	Glu	Gln	Gln
	35					40					45				
Lys	Ala	Leu	Gly	Phe	Asp	Phe	Ile	Ala	Ser	Glu	Gly	Asn	Val	Ala	Asp
50					55					60					
Trp	Asp	Ser	Thr	Lys	Thr	Ala	Phe	Asp	Lys	Val	Lys	Ser	Glu	Val	Gly
65				70					75					80	
Glu	Val	Asp	Val	Leu	Ile	Asn	Asn	Ala	Gly	Ile	Thr	Arg	Asp	Val	Val
			85					90					95		
Phe	Arg	Lys	Met	Thr	Arg	Ala	Asp	Trp	Asp	Ala	Val	Ile	Asp	Thr	Asn
		100					105					110			

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Leu Thr Ser Leu Phe Asn Val Thr Lys Gln Val Ile Asp Gly Met Ala
        115                 120                 125

Asp Arg Gly Trp Gly Arg Ile Val Asn Ile Ser Ser Val Asn Gly Gln
    130                 135                 140

Lys Gly Gln Phe Gly Gln Thr Asn Tyr Ser Thr Ala Lys Ala Gly Leu
145                 150                 155                 160

His Gly Phe Thr Met Ala Leu Ala Gln Glu Val Ala Thr Lys Gly Val
                165                 170                 175

Thr Val Asn Thr Val Ser Pro Gly Tyr Ile Ala Thr Asp Met Val Lys
            180                 185                 190

Ala Ile Arg Gln Asp Val Leu Asp Lys Ile Val Ala Thr Ile Pro Val
        195                 200                 205

Lys Arg Leu Gly Leu Pro Glu Glu Ile Ala Ser Ile Cys Ala Trp Leu
    210                 215                 220

Ser Ser Glu Glu Ser Gly Phe Ser Thr Gly Ala Asp Phe Ser Leu Asn
225                 230                 235                 240

Gly Gly Leu His Met Gly Gly Ser Met Thr Asp Val Val Ile Val Ser
                245                 250                 255

Ala Ala Arg Thr Ala Val Gly Lys Phe Gly Gly Ser Leu Ala Lys Ile
            260                 265                 270

Pro Ala Pro Glu Leu Gly Ala Val Val Ile Lys Ala Ala Leu Glu Arg
        275                 280                 285

Ala Gly Val Lys Pro Glu Gln Val Ser Glu Val Ile Met Gly Gln Val
    290                 295                 300

Leu Thr Ala Gly Ser Gly Gln Asn Pro Ala Arg Gln Ala Ala Ile Lys
305                 310                 315                 320

Ala Gly Leu Pro Ala Met Val Pro Ala Met Thr Ile Asn Lys Val Cys
                325                 330                 335

Gly Ser Gly Leu Lys Ala Val Met Leu Ala Ala Asn Ala Ile Met Ala
            340                 345                 350

Gly Asp Ala Glu Ile Val Val Ala Gly Gly Gln Glu Asn Met Ser Ala
        355                 360                 365
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Ala Pro His Val Leu Pro Gly Ser Arg Asp Gly Phe Arg Met Gly Asp
    370                 375                 380

Ala Lys Leu Val Asp Thr Met Ile Val Asp Gly Leu Trp Asp Val Tyr
385                 390                 395                 400

Asn Gln Tyr His Met Gly Ile Thr Ala Glu Asn Val Ala Lys Glu Tyr
                405                 410                 415

Gly Ile Thr Arg Glu Ala Gln Asp Glu Phe Ala Val Gly Ser Gln Asn
            420                 425                 430

Lys Ala Glu Ala Ala Gln Lys Ala Gly Lys Phe Asp Glu Glu Ile Val
        435                 440                 445

Pro Val Leu Ile Pro Gln Arg Lys Gly Asp Pro Val Ala Phe Lys Thr
    450                 455                 460

Asp Glu Phe Val Arg Gln Gly Ala Thr Leu Asp Ser Met Ser Gly Leu
465                 470                 475                 480

Lys Pro Ala Phe Asp Lys Ala Gly Thr Val Thr Ala Ala Asn Ala Ser
                485                 490                 495

Gly Leu Asn Asp Gly Ala Ala Ala Val Val Val Met Ser Ala Ala Lys
            500                 505                 510

Ala Lys Glu Leu Gly Leu Thr Pro Leu Ala Thr Ile Lys Ser Tyr Ala
        515                 520                 525

Asn Ala Gly Val Asp Pro Lys Val Met Gly Met Gly Pro Val Pro Ala
    530                 535                 540

Ser Lys Arg Ala Leu Ser Arg Ala Glu Trp Thr Pro Gln Asp Leu Asp
545                 550                 555                 560

Leu Met Glu Ile Asn Glu Ala Phe Ala Ala Gln Ala Leu Ala Val His
                565                 570                 575

Gln Gln Met Gly Trp Asp Thr Ser Lys Val Asn Val Asn Gly Gly Ala
            580                 585                 590

Ile Ala Ile Gly His Pro Ile Gly Ala Ser Gly Cys Arg Ile Leu Val
        595                 600                 605

Thr Leu Leu His Glu Met Lys Arg Arg Asp Ala Lys Lys Gly Leu Ala
    610                 615                 620
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Ser Leu Cys Ile Gly Gly Gly Met Gly Val Ala Leu Ala Val Glu Arg
625 630 635 640

Lys

<210> 20
<211> 1680
<212> DNA
<213> Pseudomonas oleovorans

<220>
<221> gene
<222> (1)..(1680)
<223> phbC1 gene

<400> 20
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aacccggtca tcggtatccg ccgcaaagac ctgttgagct cggcacgcac cgtgctgcgc 120
caggccgtgc gccaaccgct gcacagcgcc aagcatgtgg cccactttgg cctggagctg 180
aagaacgtgc tgctgggcaa gtccagcctt gccccggaaa gcgacgaccg tcgcttcaat 240
gacccggcat ggagcaacaa cccactttac cgccgctacc tgcaaaccta tctggcctgg 300
cgcaaggagc tgcaggactg gatcggcaac agcgacctgt cgccccagga catcagccgc 360
ggccagttcg tcatcaacct gatgaccgaa gccatggctc cgaccaacac cctgtccaac 420
ccggcagcag tcaaacgctt cttcgaaacc ggcggcaaga gcctgctcga tggcctgtcc 480
aacctggcca aggacctggt caacaacggt ggcatgccca gccaggtgaa catggacgcc 540
ttcgaggtgg gcaagaacct gggcaccagt gaaggcgccg tggtgtaccg caacgatgtg 600
ctggagctga tccagtacaa gcccatcacc gagcaggtgc atgcccgccc gctgctggtg 660
gtgccgccgc agatcaacaa gttctacgta ttcgacctga gcccggaaaa gagcctggca 720
cgctactgcc tgcgctcgca gcagcagacc ttcatcatca gctggcgcaa cccgaccaaa 780
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accctgctgg tcagcgtgct ggacaccacc atggacaacc aggtcgccct gttcgtcgac 1020
gagcagactt tggaggccgc caagcgccac tcctaccagg ccggtgtgct cgaaggcagc 1080
gagatggcca aggtgttcgc ctggatgcgc cccaacgacc tgatctggaa ctactgggtc 1140
aacaactacc tgctcggcaa cgagccgccg gtgttcgaca tcctgttctg gaacaacgac 1200
accacgcgcc tgccggccgc cttccacggc gacctgatcg aaatgttcaa gagcaacccg 1260
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tgcgacatct acagccttgc cggcaccaac gaccacatca ccccgtggca gtcatgctac 1380
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cagagcatcc tcaacccgcc aggcaacccc aaggcgcgct tcatgaccgg tgccgatcgc 1500
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cactggcaaa gctggctggg cgagcgtgcc ggcgagctgg aaaaggcgcc gacccgcctg 1620
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<210> 21

<211> 559
<212> PRT
<213> Pseudomonas oleovorans

<220>
<221> PEPTIDE
<222> (1)..(559)
<223> PHA Polymerase

<400> 21

Met Ser Asn Lys Asn Asn Asp Glu Leu Gln Arg Gln Ala Ser Glu Asn
1 5 10 15

Thr Leu Gly Leu Asn Pro Val Ile Gly Ile Arg Arg Lys Asp Leu Leu
20 25 30

Ser Ser Ala Arg Thr Val Leu Arg Gln Ala Val Arg Gln Pro Leu His
35 40 45

Ser Ala Lys His Val Ala His Phe Gly Leu Glu Leu Lys Asn Val Leu
50 55 60

Leu Gly Lys Ser Ser Leu Ala Pro Glu Ser Asp Asp Arg Arg Phe Asn
65 70 75 80

Asp Pro Ala Trp Ser Asn Asn Pro Leu Tyr Arg Arg Tyr Leu Gln Thr
85 90 95

Tyr Leu Ala Trp Arg Lys Glu Leu Gln Asp Trp Ile Gly Asn Ser Asp
100 105 110

Leu Ser Pro Gln Asp Ile Ser Arg Gly Gln Phe Val Ile Asn Leu Met
115 120 125

Thr Glu Ala Met Ala Pro Thr Asn Thr Leu Ser Asn Pro Ala Ala Val
130 135 140

Lys Arg Phe Phe Glu Thr Gly Gly Lys Ser Leu Leu Asp Gly Leu Ser
145 150 155 160

Asn Leu Ala Lys Asp Leu Val Asn Asn Gly Gly Met Pro Ser Gln Val
165 170 175

Asn Met Asp Ala Phe Glu Val Gly Lys Asn Leu Gly Thr Ser Glu Gly
180 185 190

Ala Val Val Tyr Arg Asn Asp Val Leu Glu Leu Ile Gln Tyr Lys Pro
195 200 205

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Ile Thr Glu Gln Val His Ala Arg Pro Leu Leu Val Val Pro Pro Gln
    210                 215                 220

Ile Asn Lys Phe Tyr Val Phe Asp Leu Ser Pro Glu Lys Ser Leu Ala
225                 230                 235                 240

Arg Tyr Cys Leu Arg Ser Gln Gln Gln Thr Phe Ile Ile Ser Trp Arg
                245                 250                 255

Asn Pro Thr Lys Ala Gln Arg Glu Trp Gly Leu Ser Thr Tyr Ile Asp
            260                 265                 270

Ala Leu Lys Glu Ala Val Asp Ala Val Leu Ala Ile Thr Gly Ser Lys
        275                 280                 285

Asp Leu Asn Met Leu Gly Ala Cys Ser Gly Gly Ile Thr Cys Thr Ala
    290                 295                 300

Leu Val Gly His Tyr Ala Ala Leu Gly Glu Asn Lys Val Asn Ala Leu
305                 310                 315                 320

Thr Leu Leu Val Ser Val Leu Asp Thr Thr Met Asp Asn Gln Val Ala
                325                 330                 335

Leu Phe Val Asp Glu Gln Thr Leu Glu Ala Ala Lys Arg His Ser Tyr
            340                 345                 350

Gln Ala Gly Val Leu Glu Gly Ser Glu Met Ala Lys Val Phe Ala Trp
        355                 360                 365

Met Arg Pro Asn Asp Leu Ile Trp Asn Tyr Trp Val Asn Asn Tyr Leu
    370                 375                 380

Leu Gly Asn Glu Pro Pro Val Phe Asp Ile Leu Phe Trp Asn Asn Asp
385                 390                 395                 400

Thr Thr Arg Leu Pro Ala Ala Phe His Gly Asp Leu Ile Glu Met Phe
                405                 410                 415

Lys Ser Asn Pro Leu Thr Arg Pro Asp Ala Leu Glu Val Cys Gly Thr
            420                 425                 430

Pro Ile Asp Leu Lys Gln Val Lys Cys Asp Ile Tyr Ser Leu Ala Gly
        435                 440                 445

Thr Asn Asp His Ile Thr Pro Trp Gln Ser Cys Tyr Arg Ser Ala His
    450                 455                 460
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Leu Phe Gly Gly Lys Ile Glu Phe Val Leu Ser Asn Ser Gly His Ile
465 470 475 480

Gln Ser Ile Leu Asn Pro Pro Gly Asn Pro Lys Ala Arg Phe Met Thr
485 490 495

Gly Ala Asp Arg Pro Gly Asp Pro Val Ala Trp Gln Glu Asn Ala Thr
500 505 510

Lys His Ala Asp Ser Trp Trp Leu His Trp Gln Ser Trp Leu Gly Glu
515 520 525

Arg Ala Gly Glu Leu Glu Lys Ala Pro Thr Arg Leu Gly Asn Arg Ala
530 535 540

Tyr Ala Ala Gly Glu Ala Ser Pro Gly Thr Tyr Val His Glu Arg
545 550 555

<210> 22
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide primer- C3 up I

<400> 22
ggaattcagg aggttttatg agtaacaaga acaacgatga gc 42

<210> 23
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide primer- C3 up II

<400> 23
cgggatccac gctcgtgaac gtaggtgccc 30

<210> 24
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide primer- C3 dw I

<400> 24
cgggatccag taacaagaac aacgatgagc 30

<210> 25
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide primer- C3 dw II

<400> 25
gctctagaag ctttcaacgc tcgtgaacgt aggtgccc 38

<210> 26
<211> 888
<212> DNA
<213> Pseudomonas putida

<220>
<221> gene
<222> (1)..(888)
<223> phaG

<400> 26
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gcctcgttcg cccagacggt acgtaacctg cacccacagt tcaacgtggt tctgttcgac 180
cagccgtatt caggcaagtc caagccgcac aaccgtcagg aacggctgat cagcaaggag 240
accgaggcgc atatcctcct tgagctgatc gagcacttcc aggcagacca cgtgatgtct 300
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gagcgcgacg agtacaccac agtcgaggat gcgcggcagt tcagcaagca tgtgggcaga 720
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tgcgagaaca cccgcaatgt catgctgggc ttcctcaagc caaccgtgcg tgaaccccgc 840
caacgttacc aacccgtgca gcaggggcag catgcatttg ccatctga 888

<210> 27

<211> 295
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: acyl ACP-CoA transferase

<220>
<221> PEPTIDE
<222> (1)..(295)

<400> 27
Met Arg Pro Glu Ile Ala Val Leu Asp Ile Gln Gly Gln Tyr Arg Val
1 5 10 15

Tyr Thr Glu Phe Tyr Arg Ala Asp Ala Ala Glu Asn Thr Ile Ile Leu
20 25 30

Ile Asn Gly Ser Leu Ala Thr Thr Ala Ser Phe Ala Gln Thr Val Arg
35 40 45

Asn Leu His Pro Gln Phe Asn Val Val Leu Phe Asp Gln Pro Tyr Ser
50 55 60

Gly Lys Ser Lys Pro His Asn Arg Gln Glu Arg Leu Ile Ser Lys Glu
65 70 75 80

Thr Glu Ala His Ile Leu Leu Glu Leu Ile Glu His Phe Gln Ala Asp
85 90 95

His Val Met Ser Phe Ser Trp Gly Gly Ala Ser Thr Leu Leu Ala Leu
100 105 110

Ala His Gln Pro Arg Tyr Val Lys Lys Ala Val Val Ser Ser Phe Ser
115 120 125

Pro Val Ile Asn Glu Pro Met Arg Asp Tyr Leu Asp Arg Gly Cys Gln
130 135 140

Tyr Leu Ala Ala Cys Asp Arg Tyr Gln Val Gly Asn Leu Val Asn Asp
145 150 155 160

Thr Ile Gly Lys His Leu Pro Ser Leu Phe Lys Arg Phe Asn Tyr Arg
165 170 175

His Val Ser Ser Leu Asp Ser His Glu Tyr Ala Gln Met His Phe His
180 185 190

Ile Asn Gln Val Leu Glu His Asp Leu Glu Arg Ala Leu Gln Gly Ala
195 200 205

Arg Asn Ile Asn Ile Pro Val Leu Phe Ile Asn Gly Glu Arg Asp Glu
210 215 220

Tyr Thr Thr Val Glu Asp Ala Arg Gln Phe Ser Lys His Val Gly Arg
225 230 235 240

Ser Gln Phe Ser Val Ile Arg Asp Ala Gly His Phe Leu Asp Met Glu
245 250 255

Asn Lys Thr Ala Cys Glu Asn Thr Arg Asn Val Met Leu Gly Phe Leu
260 265 270

Lys Pro Thr Val Arg Glu Pro Arg Gln Arg Tyr Gln Pro Val Gln Gln
275 280 285

Gly Gln His Ala Phe Ala Ile
290 295

<210> 28
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide primer- G3 dw I

<400> 28
cgggatccag gccagaaatc gctgtacttg 30

<210> 29
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide primer- G3 dw II

<400> 29
gctctagaag ctttcagatg gcaaatgcat gctgcccc 38

<210> 30

<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide primer- G3 up I

<400> 30
ggaattcagg aggttttatg aggccagaaa tcgctgtact tg 42

<210> 31
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide primer- G3 up II

<400> 31
cgggatccga tggcaaatgc atgctgcccc 30

<210> 32
<211> 2571
<212> DNA
<213> Pseudomonas putida

<220>
<221> gene
<222> (1)..(2571)
<223> phaC1-linker-phaG fusion gene

<400> 32
atgagtaaca agaacaacga tgagctgcag cggcaggcct cggaaaacac cctggggctg 60
aacccggtca tcggtatccg ccgcaaagac ctgttgagct cggcacgcac cgtgctgcgc 120
caggccgtgc gccaaccgct gcacagcgcc aagcatgtgg cccactttgg cctggagctg 180
aagaacgtgc tgctgggcaa gtccagcctt gccccggaaa gcgacgaccg tcgcttcaat 240
gacccggcat ggagcaacaa cccactttac cgccgctacc tgcaaaccta tctggcctgg 300
cgcaaggagc tgcaggactg gatcggcaac agcgacctgt cgccccagga catcagccgc 360
ggccagttcg tcatcaacct gatgaccgaa gccatggctc cgaccaacac cctgtccaac 420
ccggcagcag tcaaacgctt cttcgaaacc ggcggcaaga gcctgctcga tggcctgtcc 480
aacctggcca aggacctggt caacaacggt ggcatgccca gccaggtgaa catggacgcc 540
ttcgaggtgg gcaagaacct gggcaccagt gaaggcgccg tggtgtaccg caacgatgtg 600
ctggagctga tccagtacaa gcccatcacc gagcaggtgc atgcccgccc gctgctggtg 660
gtgccgccgc agatcaacaa gttctacgta ttcgacctga gcccggaaaa gagcctggca 720
cgctactgcc tgcgctcgca gcagcagacc ttcatcatca gctggcgcaa cccgaccaaa 780
gcccagcgcg aatggggcct gtccacctac atcgacgcgc tcaaggaggc ggtcgacgcg 840

gtgctggcga ttaccggcag caaggacctg aacatgctcg gtgcctgctc cggcggcatc 900
acctgcacgg cattggtcgg ccactatgcc gccctcggcg aaaacaaggt caatgccctg 960
accctgctgg tcagcgtgct ggacaccacc atggacaacc aggtcgccct gttcgtcgac 1020
gagcagactt tggaggccgc caagcgccac tcctaccagg ccggtgtgct cgaaggcagc 1080
gagatggcca aggtgttcgc ctggatgcgc cccaacgacc tgatctggaa ctactgggtc 1140
aacaactacc tgctcggcaa cgagccgccg gtgttcgaca tcctgttctg gaacaacgac 1200
accacgcgcc tgccggccgc cttccacggc gacctgatcg aaatgttcaa gagcaacccg 1260
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tgcgacatct acagccttgc cggcaccaac gaccacatca ccccgtggca gtcatgctac 1380
cgctcggcgc acctgttcgg cggcaagatc gagttcgtgc tgtccaacag cggccacatc 1440
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ggcaaccgtg cctatgccgc tggcgaggca tccccgggca cctacgttca cgagcgtgga 1680
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gaccagccgt attcaggcaa gtccaagccg cacaaccgtc aggaacggct gatcagcaag 1920
gagaccgagg cgcatatcct ccttgagctg atcgagcact tccaggcaga ccacgtgatg 1980
tctttttcgt ggggtggcgc aagcacgctg ctggcgctgg cgcaccagcc gcggtacgtg 2040
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gaccgtggct gccagtacct ggccgcctgc gaccgttatc aggtcggcaa cctggtcaat 2160
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agcctggaca gccacgagta cgcacagatg cacttccaca tcaaccaggt gctggagcac 2280
gacctggaac gtgcgctgca aggcgcgcgc aatatcaaca tcccggtgct gttcatcaac 2340
ggcgagcgcg acgagtacac cacagtcgag gatgcgcggc agttcagcaa gcatgtgggc 2400
agaagccagt tcagcgtgat ccgcgatgcg ggccacttcc tggacatgga gaacaagacc 2460
gcctgcgaga acacccgcaa tgtcatgctg ggcttcctca agccaaccgt gcgtgaaccc 2520
cgccaacgtt accaacccgt gcagcagggg cagcatgcat ttgccatctg a 2571

<210> 33
<211> 856
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthase Acyl
ACP-CoA Transferase Fusion Protein

<220>
<221> PEPTIDE
<222> (1)..(856)

<400> 33
Met Ser Asn Lys Asn Asn Asp Glu Leu Gln Arg Gln Ala Ser Glu Asn
1 5 10 15

Thr Leu Gly Leu Asn Pro Val Ile Gly Ile Arg Arg Lys Asp Leu Leu

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            20                  25                  30

Ser Ser Ala Arg Thr Val Leu Arg Gln Ala Val Arg Gln Pro Leu His
         35                  40                  45

Ser Ala Lys His Val Ala His Phe Gly Leu Glu Leu Lys Asn Val Leu
     50                  55                  60

Leu Gly Lys Ser Ser Leu Ala Pro Glu Ser Asp Asp Arg Arg Phe Asn
 65                  70                  75                  80

Asp Pro Ala Trp Ser Asn Asn Pro Leu Tyr Arg Arg Tyr Leu Gln Thr
                 85                  90                  95

Tyr Leu Ala Trp Arg Lys Glu Leu Gln Asp Trp Ile Gly Asn Ser Asp
            100                 105                 110

Leu Ser Pro Gln Asp Ile Ser Arg Gly Gln Phe Val Ile Asn Leu Met
        115                 120                 125

Thr Glu Ala Met Ala Pro Thr Asn Thr Leu Ser Asn Pro Ala Ala Val
    130                 135                 140

Lys Arg Phe Phe Glu Thr Gly Gly Lys Ser Leu Leu Asp Gly Leu Ser
145                 150                 155                 160

Asn Leu Ala Lys Asp Leu Val Asn Asn Gly Gly Met Pro Ser Gln Val
                165                 170                 175

Asn Met Asp Ala Phe Glu Val Gly Lys Asn Leu Gly Thr Ser Glu Gly
            180                 185                 190

Ala Val Val Tyr Arg Asn Asp Val Leu Glu Leu Ile Gln Tyr Lys Pro
        195                 200                 205

Ile Thr Glu Gln Val His Ala Arg Pro Leu Leu Val Val Pro Pro Gln
    210                 215                 220

Ile Asn Lys Phe Tyr Val Phe Asp Leu Ser Pro Glu Lys Ser Leu Ala
225                 230                 235                 240

Arg Tyr Cys Leu Arg Ser Gln Gln Gln Thr Phe Ile Ile Ser Trp Arg
                245                 250                 255

Asn Pro Thr Lys Ala Gln Arg Glu Trp Gly Leu Ser Thr Tyr Ile Asp
            260                 265                 270

Ala Leu Lys Glu Ala Val Asp Ala Val Leu Ala Ile Thr Gly Ser Lys
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275 280 285

Asp Leu Asn Met Leu Gly Ala Cys Ser Gly Gly Ile Thr Cys Thr Ala
290 295 300

Leu Val Gly His Tyr Ala Ala Leu Gly Glu Asn Lys Val Asn Ala Leu
305 310 315 320

Thr Leu Leu Val Ser Val Leu Asp Thr Thr Met Asp Asn Gln Val Ala
325 330 335

Leu Phe Val Asp Glu Gln Thr Leu Glu Ala Ala Lys Arg His Ser Tyr
340 345 350

Gln Ala Gly Val Leu Glu Gly Ser Glu Met Ala Lys Val Phe Ala Trp
355 360 365

Met Arg Pro Asn Asp Leu Ile Trp Asn Tyr Trp Val Asn Asn Tyr Leu
370 375 380

Leu Gly Asn Glu Pro Pro Val Phe Asp Ile Leu Phe Trp Asn Asn Asp
385 390 395 400

Thr Thr Arg Leu Pro Ala Ala Phe His Gly Asp Leu Ile Glu Met Phe
405 410 415

Lys Ser Asn Pro Leu Thr Arg Pro Asp Ala Leu Glu Val Cys Gly Thr
420 425 430

Pro Ile Asp Leu Lys Gln Val Lys Cys Asp Ile Tyr Ser Leu Ala Gly
435 440 445

Thr Asn Asp His Ile Thr Pro Trp Gln Ser Cys Tyr Arg Ser Ala His
450 455 460

Leu Phe Gly Gly Lys Ile Glu Phe Val Leu Ser Asn Ser Gly His Ile
465 470 475 480

Gln Ser Ile Leu Asn Pro Pro Gly Asn Pro Lys Ala Arg Phe Met Thr
485 490 495

Gly Ala Asp Arg Pro Gly Asp Pro Val Ala Trp Gln Glu Asn Ala Thr
500 505 510

Lys His Ala Asp Ser Trp Trp Leu His Trp Gln Ser Trp Leu Gly Glu
515 520 525

Arg Ala Gly Glu Leu Glu Lys Ala Pro Thr Arg Leu Gly Asn Arg Ala

530 535 540

Tyr Ala Ala Gly Glu Ala Ser Pro Gly Thr Tyr Val His Glu Arg Gly
545 550 555 560

Phe Met Arg Pro Glu Ile Ala Val Leu Asp Ile Gln Gly Gln Tyr Arg
565 570 575

Val Tyr Thr Glu Phe Tyr Arg Ala Asp Ala Ala Glu Asn Thr Ile Ile
580 585 590

Leu Ile Asn Gly Ser Leu Ala Thr Thr Ala Ser Phe Ala Gln Thr Val
595 600 605

Arg Asn Leu His Pro Gln Phe Asn Val Val Leu Phe Asp Gln Pro Tyr
610 615 620

Ser Gly Lys Ser Lys Pro His Asn Arg Gln Glu Arg Leu Ile Ser Lys
625 630 635 640

Glu Thr Glu Ala His Ile Leu Leu Glu Leu Ile Glu His Phe Gln Ala
645 650 655

Asp His Val Met Ser Phe Ser Trp Gly Gly Ala Ser Thr Leu Leu Ala
660 665 670

Leu Ala His Gln Pro Arg Tyr Val Lys Lys Ala Val Val Ser Ser Phe
675 680 685

Ser Pro Val Ile Asn Glu Pro Met Arg Asp Tyr Leu Asp Arg Gly Cys
690 695 700

Gln Tyr Leu Ala Ala Cys Asp Arg Tyr Gln Val Gly Asn Leu Val Asn
705 710 715 720

Asp Thr Ile Gly Lys His Leu Pro Ser Leu Phe Lys Arg Phe Asn Tyr
725 730 735

Arg His Val Ser Ser Leu Asp Ser His Glu Tyr Ala Gln Met His Phe
740 745 750

His Ile Asn Gln Val Leu Glu His Asp Leu Glu Arg Ala Leu Gln Gly
755 760 765

Ala Arg Asn Ile Asn Ile Pro Val Leu Phe Ile Asn Gly Glu Arg Asp
770 775 780

Glu Tyr Thr Thr Val Glu Asp Ala Arg Gln Phe Ser Lys His Val Gly

785 790 795 800

Arg Ser Gln Phe Ser Val Ile Arg Asp Ala Gly His Phe Leu Asp Met
805 810 815

Glu Asn Lys Thr Ala Cys Glu Asn Thr Arg Asn Val Met Leu Gly Phe
820 825 830

Leu Lys Pro Thr Val Arg Glu Pro Arg Gln Arg Tyr Gln Pro Val Gln
835 840 845

Gln Gly Gln His Ala Phe Ala Ile
850 855

<210> 34
<211> 2571
<212> DNA
<213> Pseudomonas putida

<220>
<221> gene
<222> (1)..(2571)
<223> phaG-linker-phaC1 fusion gene

<400> 34
atgaggccag aaatcgctgt acttgatatc caaggtcagt atcgggttta cacggagttc 60
tatcgcgcgg atgcggccga aaacacgatc atcctgatca acggctcgct ggccaccacg 120
gcctcgttcg cccagacggt acgtaacctg cacccacagt tcaacgtggt tctgttcgac 180
cagccgtatt caggcaagtc caagccgcac aaccgtcagg aacggctgat cagcaaggag 240
accgaggcgc atatcctcct tgagctgatc gagcacttcc aggcagacca cgtgatgtct 300
ttttcgtggg gtggcgcaag cacgctgctg gcgctggcgc accagccgcg gtacgtgaag 360
aaggcagtgg tgagttcgtt ctcgccagtg atcaacgagc cgatgcgcga ctatctggac 420
cgtggctgcc agtacctggc cgcctgcgac cgttatcagg tcggcaacct ggtcaatgac 480
accatcggca agcacttgcc gtcgctgttc aaacgcttca actaccgcca tgtgagcagc 540
ctggacagcc acgagtacgc acagatgcac ttccacatca accaggtgct ggagcacgac 600
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gagcgcgacg agtacaccac agtcgaggat gcgcggcagt tcagcaagca tgtgggcaga 720
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aagaacaacg atgagctgca gcggcaggcc tcggaaaaca ccctggggct gaacccggtc 960
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ctgctgggca agtccagcct tgccccggaa agcgacgacc gtcgcttcaa tgacccggca 1140
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ctgcaggact ggatcggcaa cagcgacctg tcgccccagg acatcagccg cggccagttc 1260
gtcatcaacc tgatgaccga agccatggct ccgaccaaca ccctgtccaa cccggcagca 1320

gtcaaacgct tcttcgaaac cggcggcaag agcctgctcg atggcctgtc caacctggcc 1380
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atccagtaca agcccatcac cgagcaggtg catgcccgcc cgctgctggt ggtgccgccg 1560
cagatcaaca agttctacgt attcgacctg agcccggaaa agagcctggc acgctactgc 1620
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tacagccttg ccggcaccaa cgaccacatc accccgtggc agtcatgcta ccgctcggcg 2280
cacctgttcg gcggcaagat cgagttcgtg ctgtccaaca gcggccacat ccagagcatc 2340
ctcaacccgc caggcaaccc caaggcgcgc ttcatgaccg gtgccgatcg cccgggtgac 2400
ccggtggcct ggcaggaaaa cgccaccaag catgccgact cctggtggct gcactggcaa 2460
agctggctgg gcgagcgtgc cggcgagctg gaaaaggcgc cgacccgcct gggcaaccgt 2520
gcctatgccg ctggcgaggc atccccgggc acctacgttc acgagcgttg a 2571

<210> 35
<211> 856
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Acyl ACP-CoA
Transferase Synthase Fusion Protein

<220>
<221> PEPTIDE
<222> (1)..(856)

<400> 35
Met Arg Pro Glu Ile Ala Val Leu Asp Ile Gln Gly Gln Tyr Arg Val
1 5 10 15

Tyr Thr Glu Phe Tyr Arg Ala Asp Ala Ala Glu Asn Thr Ile Ile Leu
20 25 30

Ile Asn Gly Ser Leu Ala Thr Thr Ala Ser Phe Ala Gln Thr Val Arg
35 40 45

Asn Leu His Pro Gln Phe Asn Val Val Leu Phe Asp Gln Pro Tyr Ser
50 55 60

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Gly Lys Ser Lys Pro His Asn Arg Gln Glu Arg Leu Ile Ser Lys Glu
 65                  70                  75                  80

Thr Glu Ala His Ile Leu Leu Glu Leu Ile Glu His Phe Gln Ala Asp
                85                  90                  95

His Val Met Ser Phe Ser Trp Gly Gly Ala Ser Thr Leu Leu Ala Leu
            100                 105                 110

Ala His Gln Pro Arg Tyr Val Lys Lys Ala Val Val Ser Ser Phe Ser
        115                 120                 125

Pro Val Ile Asn Glu Pro Met Arg Asp Tyr Leu Asp Arg Gly Cys Gln
    130                 135                 140

Tyr Leu Ala Ala Cys Asp Arg Tyr Gln Val Gly Asn Leu Val Asn Asp
145                 150                 155                 160

Thr Ile Gly Lys His Leu Pro Ser Leu Phe Lys Arg Phe Asn Tyr Arg
                165                 170                 175

His Val Ser Ser Leu Asp Ser His Glu Tyr Ala Gln Met His Phe His
            180                 185                 190

Ile Asn Gln Val Leu Glu His Asp Leu Glu Arg Ala Leu Gln Gly Ala
        195                 200                 205

Arg Asn Ile Asn Ile Pro Val Leu Phe Ile Asn Gly Glu Arg Asp Glu
    210                 215                 220

Tyr Thr Thr Val Glu Asp Ala Arg Gln Phe Ser Lys His Val Gly Arg
225                 230                 235                 240

Ser Gln Phe Ser Val Ile Arg Asp Ala Gly His Phe Leu Asp Met Glu
                245                 250                 255

Asn Lys Thr Ala Cys Glu Asn Thr Arg Asn Val Met Leu Gly Phe Leu
            260                 265                 270

Lys Pro Thr Val Arg Glu Pro Arg Gln Arg Tyr Gln Pro Val Gln Gln
        275                 280                 285

Gly Gln His Ala Phe Ala Ile Gly Ser Met Ser Asn Lys Asn Asn Asp
    290                 295                 300

Glu Leu Gln Arg Gln Ala Ser Glu Asn Thr Leu Gly Leu Asn Pro Val
305                 310                 315                 320
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Ile Gly Ile Arg Arg Lys Asp Leu Leu Ser Ser Ala Arg Thr Val Leu
325 330 335

Arg Gln Ala Val Arg Gln Pro Leu His Ser Ala Lys His Val Ala His
340 345 350

Phe Gly Leu Glu Leu Lys Asn Val Leu Leu Gly Lys Ser Ser Leu Ala
355 360 365

Pro Glu Ser Asp Asp Arg Arg Phe Asn Asp Pro Ala Trp Ser Asn Asn
370 375 380

Pro Leu Tyr Arg Arg Tyr Leu Gln Thr Tyr Leu Ala Trp Arg Lys Glu
385 390 395 400

Leu Gln Asp Trp Ile Gly Asn Ser Asp Leu Ser Pro Gln Asp Ile Ser
405 410 415

Arg Gly Gln Phe Val Ile Asn Leu Met Thr Glu Ala Met Ala Pro Thr
420 425 430

Asn Thr Leu Ser Asn Pro Ala Ala Val Lys Arg Phe Phe Glu Thr Gly
435 440 445

Gly Lys Ser Leu Leu Asp Gly Leu Ser Asn Leu Ala Lys Asp Leu Val
450 455 460

Asn Asn Gly Gly Met Pro Ser Gln Val Asn Met Asp Ala Phe Glu Val
465 470 475 480

Gly Lys Asn Leu Gly Thr Ser Glu Gly Ala Val Val Tyr Arg Asn Asp
485 490 495

Val Leu Glu Leu Ile Gln Tyr Lys Pro Ile Thr Glu Gln Val His Ala
500 505 510

Arg Pro Leu Leu Val Val Pro Pro Gln Ile Asn Lys Phe Tyr Val Phe
515 520 525

Asp Leu Ser Pro Glu Lys Ser Leu Ala Arg Tyr Cys Leu Arg Ser Gln
530 535 540

Gln Gln Thr Phe Ile Ile Ser Trp Arg Asn Pro Thr Lys Ala Gln Arg
545 550 555 560

Glu Trp Gly Leu Ser Thr Tyr Ile Asp Ala Leu Lys Glu Ala Val Asp
565 570 575

Ala Val Leu Ala Ile Thr Gly Ser Lys Asp Leu Asn Met Leu Gly Ala
            580   585   590

Cys Ser Gly Gly Ile Thr Cys Thr Ala Leu Val Gly His Tyr Ala Ala
        595   600   605

Leu Gly Glu Asn Lys Val Asn Ala Leu Thr Leu Leu Val Ser Val Leu
    610   615   620

Asp Thr Thr Met Asp Asn Gln Val Ala Leu Phe Val Asp Glu Gln Thr
625   630   635   640

Leu Glu Ala Ala Lys Arg His Ser Tyr Gln Ala Gly Val Leu Glu Gly
                645   650   655

Ser Glu Met Ala Lys Val Phe Ala Trp Met Arg Pro Asn Asp Leu Ile
            660   665   670

Trp Asn Tyr Trp Val Asn Asn Tyr Leu Leu Gly Asn Glu Pro Pro Val
        675   680   685

Phe Asp Ile Leu Phe Trp Asn Asn Asp Thr Thr Arg Leu Pro Ala Ala
    690   695   700

Phe His Gly Asp Leu Ile Glu Met Phe Lys Ser Asn Pro Leu Thr Arg
705   710   715   720

Pro Asp Ala Leu Glu Val Cys Gly Thr Pro Ile Asp Leu Lys Gln Val
                725   730   735

Lys Cys Asp Ile Tyr Ser Leu Ala Gly Thr Asn Asp His Ile Thr Pro
            740   745   750

Trp Gln Ser Cys Tyr Arg Ser Ala His Leu Phe Gly Gly Lys Ile Glu
        755   760   765

Phe Val Leu Ser Asn Ser Gly His Ile Gln Ser Ile Leu Asn Pro Pro
    770   775   780

Gly Asn Pro Lys Ala Arg Phe Met Thr Gly Ala Asp Arg Pro Gly Asp
785   790   795   800

Pro Val Ala Trp Gln Glu Asn Ala Thr Lys His Ala Asp Ser Trp Trp
                805   810   815

Leu His Trp Gln Ser Trp Leu Gly Glu Arg Ala Gly Glu Leu Glu Lys
            820   825   830

Ala Pro Thr Arg Leu Gly Asn Arg Ala Tyr Ala Ala Gly Glu Ala Ser
835 840 845

Pro Gly Thr Tyr Val His Glu Arg
850 855

<210> 36
<211> 1731
<212> DNA
<213> Zoogloea ramigera

<220>
<221> gene
<222> (1)..(1731)
<223> phbC gene

<400> 36
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atgttgcagg atatcggcgt tgcgctcaaa ccggaagcga tggagcagct gaaaaacgat 180
tatctgcgtg acttcaccgc gttgtggcag gatttttttgg ctggcaaggc gccagccgtc 240
cagcgaccgc gcttcagctc ggcagcctgg cagggcaatc cgatgtcggc cttcaatgcc 300
gcatcttacc tgctcaacgc caaattcctc agtgccatgg tggaggcggt ggacaccgca 360
ccccagcaaa agcagaaaat acgctttgcc gtgcagcagg tgattgatgc catgtcgccc 420
gcgaacttcc tcgccaccaa cccggaagcg cagcaaaaac tgattgaaac caagggcgag 480
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aacggccata tctcgctgtc ggacgaatcg gcctttgaag tgggccgcaa cctggccatt 600
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acgccgacgg tcagccagcg cccgctgttg atggtgccgc cgtgcatcaa caagttctac 720
atcctcgacc tgcaaccgga aaattcgctg gtgcgctacg cggtggagca gggcaacacc 780
gtgttcctga tctcgtggag caatccggac aagtcgctgg ccggcaccac ctgggacgac 840
tacgtggagc agggcgtgat cgaagcgatc cgcatcgtcc aggacgtcag cggccaggac 900
aagctgaaca tgttcggctt ctgcgtgggc ggcaccatcg ttgccaccgc actggcggta 960
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ttcagcgaca ccgggtgctc gacgtcttgt cgagaaaccc aggtcgcgct gcgtgaacag 1080
caattgcgcg atggcggcct gatgccgggc cgtgacctgg cctcgacctt ctcgagcctg 1140
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gcggcgtttg acctgctgtt ctggaattcg gacagcacca atttgccggg cccgatgttc 1260
tgctggtacc tgcgcaacac ctacctggaa aacagcctga aagtgccggg caagctgacg 1320
gtggccggcg aaaagatcga cctcggcctg atcgacgccc cggccttcat ctacggttcg 1380
cgcgaagacc acatcgtgcc gtggatgtcg gcgtacggtt cgctcgacat cctgaaccag 1440
ggcaagccgg gcgccaaccg cttcgtgctg ggcgcgtccg gccatatcgc cggcgtgatc 1500
aactcggtgg ccaagaacaa gcgcacgtac tggatcaacg acggtggcgc cgccgatgcc 1560
caggcctggt tcgatggcgc gcaggaagtg ccgggcagct ggtggccgca atgggccggg 1620
ttcctgaccc agcatggcgg caagaaggtc aagcccaagg ccaagcccgg caacgcccgc 1680
tacaccgcga tcgaggcggc gcccggccgt tacgtcaaag ccaagggctg a 1731

<210> 37
<211> 576
<212> PRT
<213> Zoogloea ramigera

<220>
<221> PEPTIDE
<222> (1)..(576)
<223> synthase

<400> 37
Met Asn Leu Pro Asp Pro Gln Ala Ile Ala Asn Ala Trp Met Ser Gln
1 5 10 15

Val Gly Asp Pro Ser Gln Trp Gln Ser Trp Phe Ser Lys Ala Pro Thr
20 25 30

Thr Glu Ala Asn Pro Met Ala Thr Met Leu Gln Asp Ile Gly Val Ala
35 40 45

Leu Lys Pro Glu Ala Met Glu Gln Leu Lys Asn Asp Tyr Leu Arg Asp
50 55 60

Phe Thr Ala Leu Trp Gln Asp Phe Leu Ala Gly Lys Ala Pro Ala Val
65 70 75 80

Gln Arg Pro Arg Phe Ser Ser Ala Ala Trp Gln Gly Asn Pro Met Ser
85 90 95

Ala Phe Asn Ala Ala Ser Tyr Leu Leu Asn Ala Lys Phe Leu Ser Ala
100 105 110

Met Val Glu Ala Val Asp Thr Ala Pro Gln Gln Lys Gln Lys Ile Arg
115 120 125

Phe Ala Val Gln Gln Val Ile Asp Ala Met Ser Pro Ala Asn Phe Leu
130 135 140

Ala Thr Asn Pro Glu Ala Gln Gln Lys Leu Ile Glu Thr Lys Gly Glu
145 150 155 160

Ser Leu Thr Arg Gly Leu Val Asn Met Leu Gly Asp Ile Asn Met Leu
165 170 175

Gly Asp Ile Asn Asn Gly His Ile Ser Leu Ser Asp Glu Ser Ala Phe
180 185 190

Glu Val Gly Arg Asn Leu Ala Ile Thr Pro Gly Thr Val Ile Tyr Glu

195 200 205

Asn Pro Leu Phe Gln Leu Ile Gln Tyr Thr Pro Thr Thr Pro Thr Val
210 215 220

Ser Gln Arg Pro Leu Leu Met Val Pro Pro Cys Ile Asn Lys Phe Tyr
225 230 235 240

Ile Leu Asp Leu Gln Pro Glu Asn Ser Leu Val Arg Tyr Ala Val Glu
245 250 255

Gln Gly Asn Thr Val Phe Leu Ile Ser Trp Ser Asn Pro Asp Lys Ser
260 265 270

Leu Ala Gly Thr Thr Trp Asp Asp Tyr Val Glu Gln Gly Val Ile Glu
275 280 285

Ala Ile Arg Ile Val Gln Asp Val Ser Gly Gln Asp Lys Leu Asn Met
290 295 300

Phe Gly Phe Cys Val Gly Gly Thr Ile Val Ala Thr Ala Leu Ala Val
305 310 315 320

Leu Ala Ala Arg Gly Gln His Pro Ala Ala Ser Leu Thr Leu Leu Thr
325 330 335

Thr Phe Leu Asp Phe Ser Asp Thr Gly Cys Ser Thr Ser Cys Arg Glu
340 345 350

Thr Gln Val Ala Leu Arg Glu Gln Gln Leu Arg Asp Gly Gly Leu Met
355 360 365

Pro Gly Arg Asp Leu Ala Ser Thr Phe Ser Ser Leu Arg Pro Asn Asp
370 375 380

Leu Val Trp Asn Tyr Val Gln Ser Asn Tyr Leu Lys Gly Asn Glu Pro
385 390 395 400

Ala Ala Phe Asp Leu Leu Phe Trp Asn Ser Asp Ser Thr Asn Leu Pro
405 410 415

Gly Pro Met Phe Cys Trp Tyr Leu Arg Asn Thr Tyr Leu Glu Asn Ser
420 425 430

Leu Lys Val Pro Gly Lys Leu Thr Val Ala Gly Glu Lys Ile Asp Leu
435 440 445

Gly Leu Ile Asp Ala Pro Ala Phe Ile Tyr Gly Ser Arg Glu Asp His

450 455 460

Ile Val Pro Trp Met Ser Ala Tyr Gly Ser Leu Asp Ile Leu Asn Gln
465 470 475 480

Gly Lys Pro Gly Ala Asn Arg Phe Val Leu Gly Ala Ser Gly His Ile
485 490 495

Ala Gly Val Ile Asn Ser Val Ala Lys Asn Lys Arg Thr Tyr Trp Ile
500 505 510

Asn Asp Gly Gly Ala Ala Asp Ala Gln Ala Trp Phe Asp Gly Ala Gln
515 520 525

Glu Val Pro Gly Ser Trp Trp Pro Gln Trp Ala Gly Phe Leu Thr Gln
530 535 540

His Gly Gly Lys Lys Val Lys Pro Lys Ala Lys Pro Gly Asn Ala Arg
545 550 555 560

Tyr Thr Ala Ile Glu Ala Ala Pro Gly Arg Tyr Val Lys Ala Lys Gly
565 570 575

<210> 38
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide primer- C5 up I

<400> 38
ggagctcagg aggttttatg agtaacaaga acaacgatga gc 42

<210> 39
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide primer-C5 up II

<400> 39
cgggatccgc ccttggcttt gacgtaacgg 30

<210> 40
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide primer- C5 dw I

<400> 40
cgggatccag taacaagaac aacgatgagc 30

<210> 41
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide primer- C5 dw II

<400> 41
gctctagaag ctttcagccc ttggctttga cgtaacgg 38

<210> 42
<211> 405
<212> DNA
<213> Aeromonas caviae

<220>
<221> gene
<222> (1)..(405)
<223> phbJ gene

<400> 42
atgagcgcac aatccctgga agtaggccag aaggcccgtc tcagcaagcg gttcggggcg 60
gcggaggtag ccgccttcgc cgcgctctcg gaggacttca acccccctgca cctggacccg 120
gccttcgccg ccaccacggc gttcgagcgg cccatagtcc acggcatgct gctcgccagc 180
ctcttctccg ggctgctggg ccagcagttg ccgggcaagg ggagcatcta tctgggtcaa 240
agcctcagct tcaagctgcc ggtctttgtc ggggacgagg tgacggccga ggtggaggtg 300
accgcccttc gcgaggacaa gcccatcgcc accctgacca cccgcatctt cacccaaggc 360
ggcgccctcg ccgtgacggg ggaagccgtg gtcaagctgc cttaa 405

<210> 43
<211> 134

<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: (R) specific
enoyl-CoA transferase

<220>
<221> PEPTIDE
<222> (1)..(134)

<400> 43
Met Ser Ala Gln Ser Leu Glu Val Gly Gln Lys Ala Arg Leu Ser Lys
1 5 10 15

Arg Phe Gly Ala Ala Glu Val Ala Ala Phe Ala Ala Leu Ser Glu Asp
20 25 30

Phe Asn Pro Leu His Leu Asp Pro Ala Phe Ala Ala Thr Thr Ala Phe
35 40 45

Glu Arg Pro Ile Val His Gly Met Leu Leu Ala Ser Leu Phe Ser Gly
50 55 60

Leu Leu Gly Gln Gln Leu Pro Gly Lys Gly Ser Ile Tyr Leu Gly Gln
65 70 75 80

Ser Leu Ser Phe Lys Leu Pro Val Phe Val Gly Asp Glu Val Thr Ala
85 90 95

Glu Val Glu Val Thr Ala Leu Arg Glu Asp Lys Pro Ile Ala Thr Leu
100 105 110

Thr Thr Arg Ile Phe Thr Gln Gly Gly Ala Leu Ala Val Thr Gly Glu
115 120 125

Ala Val Val Lys Leu Pro
130

<210> 44
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide primer- J12 dw I

<400> 44
cgggatccag cgcacaatcc ctggaagtag 30

<210> 45
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide primer-J12 dw II

<400> 45
gctctagaag cttttaaggc agcttgacca cggcttcc 38

<210> 46
<211> 43
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: J12 up I

<400> 46
aggagctcag gaggttttat gagcgcacaa tccctggaag tag 43

<210> 47
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide primer- J12 up II

<400> 47
cgggatccag gcagcttgac cacggcttcc 30

<210> 48
<211> 2139
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Zoogloea
ramigera and Aeromonas caviae phaC-linker-phbJ
fusion gene

<220>
<221> gene
<222> (1)..(2139)

<400> 48
atgaatttgc ccgatccgca agccattgcc aacgcctgga tgtcccaggt gggcgacccc 60
agccaatggc aatcctggtt cagcaaggcg cccaccaccg aggcgaaccc gatggccacc 120
atgttgcagg atatcggcgt tgcgctcaaa ccggaagcga tggagcagct gaaaaacgat 180
tatctgcgtg acttcaccgc gttgtggcag gatttttgg ctggcaaggc gccagccgtc 240
cagcgaccgc gcttcagctc ggcagcctgg cagggcaatc cgatgtcggc cttcaatgcc 300
gcatcttacc tgctcaacgc caaattcctc agtgccatgg tggaggcggt ggacaccgca 360
ccccagcaaa agcagaaaat acgctttgcc gtgcagcagg tgattgatgc catgtcgccc 420
gcgaacttcc tcgccaccaa cccggaagcg cagcaaaaac tgattgaaac caagggcgag 480
agcctgacgc gtggcctggt caatatgctg ggcgatatca atatgctggg cgatatcaac 540
aacggccata tctcgctgtc ggacgaatcg gcctttgaag tgggccgcaa cctggccatt 600
accccgggca ccgtgattta cgaaaatccg ctgttccagc tgatccagta cacgccgacc 660
acgccgacgg tcagccagcg cccgctgttg atggtgccgc cgtgcatcaa caagttctac 720
atcctcgacc tgcaaccgga aaattcgctg gtgcgctacg cggtggagca gggcaacacc 780
gtgttcctga tctcgtggag caatccggac aagtcgctgg ccggcaccac ctgggacgac 840
tacgtggagc agggcgtgat cgaagcgatc cgcatcgtcc aggacgtcag cggccaggac 900
aagctgaaca tgttcggctt ctgcgtgggc ggcaccatcg ttgccaccgc actggcggta 960
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caattgcgcg atggcggcct gatgccgggc cgtgacctgg cctcgacctt ctcgagcctg 1140
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gcggcgtttg acctgctgtt ctggaattcg gacagcacca atttgccggg cccgatgttc 1260
tgctggtacc tgcgcaacac ctacctggaa aacagcctga aagtgccggg caagctgacg 1320
gtggccggcg aaaagatcga cctcggcctg atcgacgccc cggccttcat ctacggttcg 1380
cgcgaagacc acatcgtgcc gtggatgtcg gcgtacggtt cgctcgacat cctgaaccag 1440
ggcaagccgg gcgccaaccg cttcgtgctg ggcgcgtccg gccatatcgc cggcgtgatc 1500
aactcggtgg ccaagaacaa gcgcacgtac tggatcaacg acggtggcgc cgccgatgcc 1560
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tacaccgcga tcgaggcggc gcccggccgt tacgtcaaag ccaagggcgg atccatgagc 1740
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gtagccgcct tcgccgcgct ctcggaggac ttcaaccccc tgcacctgga cccggccttc 1860
gccgccacca cggcgttcga gcggcccata gtccacggca tgctgctcgc cagcctcttc 1920
tccgggctgc tgggccagca gttgccgggc aaggggagca tctatctggg tcaaagcctc 1980
agcttcaagc tgccggtctt tgtcggggac gaggtgacgg ccgaggtgga ggtgaccgcc 2040
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ctcgccgtga cgggggaagc cgtggtcaag ctgccttaa 2139

<210> 49
<211> 712
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthase (R)
specific enoyl-CoA transferase Fusion Protein

<220>
<221> PEPTIDE
<222> (1)..(712)

<400> 49

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Met Asn Leu Pro Asp Pro Gln Ala Ile Ala Asn Ala Trp Met Ser Gln
  1               5                  10                  15

Val Gly Asp Pro Ser Gln Trp Gln Ser Trp Phe Ser Lys Ala Pro Thr
             20                  25                  30

Thr Glu Ala Asn Pro Met Ala Thr Met Leu Gln Asp Ile Gly Val Ala
         35                  40                  45

Leu Lys Pro Glu Ala Met Glu Gln Leu Lys Asn Asp Tyr Leu Arg Asp
     50                  55                  60

Phe Thr Ala Leu Trp Gln Asp Phe Leu Ala Gly Lys Ala Pro Ala Val
 65                  70                  75                  80

Gln Arg Pro Arg Phe Ser Ser Ala Ala Trp Gln Gly Asn Pro Met Ser
                 85                  90                  95

Ala Phe Asn Ala Ala Ser Tyr Leu Leu Asn Ala Lys Phe Leu Ser Ala
            100                 105                 110

Met Val Glu Ala Val Asp Thr Ala Pro Gln Gln Lys Gln Lys Ile Arg
        115                 120                 125

Phe Ala Val Gln Gln Val Ile Asp Ala Met Ser Pro Ala Asn Phe Leu
    130                 135                 140

Ala Thr Asn Pro Glu Ala Gln Gln Lys Leu Ile Glu Thr Lys Gly Glu
145                 150                 155                 160

Ser Leu Thr Arg Gly Leu Val Asn Met Leu Gly Asp Ile Asn Met Leu
                165                 170                 175

Gly Asp Ile Asn Asn Gly His Ile Ser Leu Ser Asp Glu Ser Ala Phe
            180                 185                 190

Glu Val Gly Arg Asn Leu Ala Ile Thr Pro Gly Thr Val Ile Tyr Glu
        195                 200                 205
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Asn Pro Leu Phe Gln Leu Ile Gln Tyr Thr Pro Thr Thr Pro Thr Val
    210                 215                 220

Ser Gln Arg Pro Leu Leu Met Val Pro Pro Cys Ile Asn Lys Phe Tyr
225                 230                 235                 240

Ile Leu Asp Leu Gln Pro Glu Asn Ser Leu Val Arg Tyr Ala Val Glu
                245                 250                 255

Gln Gly Asn Thr Val Phe Leu Ile Ser Trp Ser Asn Pro Asp Lys Ser
            260                 265                 270

Leu Ala Gly Thr Thr Trp Asp Asp Tyr Val Glu Gln Gly Val Ile Glu
        275                 280                 285

Ala Ile Arg Ile Val Gln Asp Val Ser Gly Gln Asp Lys Leu Asn Met
    290                 295                 300

Phe Gly Phe Cys Val Gly Gly Thr Ile Val Ala Thr Ala Leu Ala Val
305                 310                 315                 320

Leu Ala Ala Arg Gly Gln His Pro Ala Ala Ser Leu Thr Leu Leu Thr
                325                 330                 335

Thr Phe Leu Asp Phe Ser Asp Thr Gly Cys Ser Thr Ser Cys Arg Glu
            340                 345                 350

Thr Gln Val Ala Leu Arg Glu Gln Gln Leu Arg Asp Gly Gly Leu Met
        355                 360                 365

Pro Gly Arg Asp Leu Ala Ser Thr Phe Ser Ser Leu Arg Pro Asn Asp
    370                 375                 380

Leu Val Trp Asn Tyr Val Gln Ser Asn Tyr Leu Lys Gly Asn Glu Pro
385                 390                 395                 400

Ala Ala Phe Asp Leu Leu Phe Trp Asn Ser Asp Ser Thr Asn Leu Pro
                405                 410                 415

Gly Pro Met Phe Cys Trp Tyr Leu Arg Asn Thr Tyr Leu Glu Asn Ser
            420                 425                 430

Leu Lys Val Pro Gly Lys Leu Thr Val Ala Gly Glu Lys Ile Asp Leu
        435                 440                 445

Gly Leu Ile Asp Ala Pro Ala Phe Ile Tyr Gly Ser Arg Glu Asp His
    450                 455                 460
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Ile Val Pro Trp Met Ser Ala Tyr Gly Ser Leu Asp Ile Leu Asn Gln
465 470 475 480

Gly Lys Pro Gly Ala Asn Arg Phe Val Leu Gly Ala Ser Gly His Ile
485 490 495

Ala Gly Val Ile Asn Ser Val Ala Lys Asn Lys Arg Thr Tyr Trp Ile
500 505 510

Asn Asp Gly Gly Ala Ala Asp Ala Gln Ala Trp Phe Asp Gly Ala Gln
515 520 525

Glu Val Pro Gly Ser Trp Trp Pro Gln Trp Ala Gly Phe Leu Thr Gln
530 535 540

His Gly Gly Lys Lys Val Lys Pro Lys Ala Lys Pro Gly Asn Ala Arg
545 550 555 560

Tyr Thr Ala Ile Glu Ala Ala Pro Gly Arg Tyr Val Lys Ala Lys Gly
565 570 575

Gly Ser Met Ser Ala Gln Ser Leu Glu Val Gly Gln Lys Ala Arg Leu
580 585 590

Ser Lys Arg Phe Gly Ala Ala Glu Val Ala Ala Phe Ala Ala Leu Ser
595 600 605

Glu Asp Phe Asn Pro Leu His Leu Asp Pro Ala Phe Ala Ala Thr Thr
610 615 620

Ala Phe Glu Arg Pro Ile Val His Gly Met Leu Leu Ala Ser Leu Phe
625 630 635 640

Ser Gly Leu Leu Gly Gln Gln Leu Pro Gly Lys Gly Ser Ile Tyr Leu
645 650 655

Gly Gln Ser Leu Ser Phe Lys Leu Pro Val Phe Val Gly Asp Glu Val
660 665 670

Thr Ala Glu Val Glu Val Thr Ala Leu Arg Glu Asp Lys Pro Ile Ala
675 680 685

Thr Leu Thr Thr Arg Ile Phe Thr Gln Gly Gly Ala Leu Ala Val Thr
690 695 700

Gly Glu Ala Val Val Lys Leu Pro
705 710

<210> 50
<211> 2139
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Aeromonas caviae and Zoogloea ramigera phbJ-linker-phaC fusion gene

<220>
<221> gene
<222> (1)..(2139)

<400> 50
atgagcgcac aatccctgga agtaggccag aaggcccgtc tcagcaagcg gttcggggcg 60
gcggaggtag ccgccttcgc cgcgctctcg gaggacttca acccccctgca cctggacccg 120
gccttcgccg ccaccacggc gttcgagcgg cccatagtcc acggcatgct gctcgccagc 180
ctcttctccg ggctgctggg ccagcagttg ccgggcaagg ggagcatcta tctgggtcaa 240
agcctcagct tcaagctgcc ggtctttgtc ggggacgagg tgacggccga ggtggaggtg 300
accgcccttc gcgaggacaa gcccatcgcc accctgacca cccgcatctt cacccaaggc 360
ggcgccctcg ccgtgacggg ggaagccgtg gtcaagctgc ctggatccat gaatttgccc 420
gatccgcaag ccattgccaa cgcctggatg tcccaggtgg gcgaccccag ccaatggcaa 480
tcctggttca gcaaggcgcc caccaccgag gcgaacccga tggccaccat gttgcaggat 540
atcggcgttg cgctcaaacc ggaagcgatg gagcagctga aaaacgatta tctgcgtgac 600
ttcaccgcgt tgtggcagga ttttttggct ggcaaggcgc cagccgtcca gcgaccgcgc 660
ttcagctcgg cagcctggca gggcaatccg atgtcggcct tcaatgccgc atcttacctg 720
ctcaacgcca aattcctcag tgccatggtg gaggcggtgg acaccgcacc ccagcaaaag 780
cagaaaatac gctttgccgt gcagcaggtg attgatgcca tgtcgcccgc gaacttcctc 840
gccaccaacc cggaagcgca gcaaaaactg attgaaacca agggcgagag cctgacgcgt 900
ggcctggtca atatgctggg cgatatcaat atgctgggcg atatcaacaa cggccatatc 960
tcgctgtcgg acgaatcggc ctttgaagtg ggccgcaacc tggccattac cccgggcacc 1020
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agccagcgcc cgctgttgat ggtgccgccg tgcatcaaca agttctacat cctcgacctg 1140
caaccggaaa attcgctggt gcgctacgcg gtggagcagg gcaacaccgt gttcctgatc 1200
tcgtggagca atccggacaa gtcgctggcc ggcaccacct gggacgacta cgtggagcag 1260
ggcgtgatcg aagcgatccg catcgtccag gacgtcagcg gccaggacaa gctgaacatg 1320
ttcggcttct gcgtgggcgg caccatcgtt gccaccgcac tggcggtact ggcggcgcgt 1380
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ggcggcctga tgccgggccg tgacctggcc tcgaccttct cgagcctgcg tccgaacgac 1560
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ctgctgttct ggaattcgga cagcaccaat ttgccgggcc cgatgttctg ctggtacctg 1680
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gccaaccgct tcgtgctggg cgcgtccggc catatcgccg gcgtgatcaa ctcggtggcc 1920

aagaacaagc gcacgtactg gatcaacgac ggtggcgccg ccgatgccca ggcctggttc 1980
gatggcgcgc aggaagtgcc gggcagctgg tggccgcaat gggccgggtt cctgacccag 2040
catggcggca agaaggtcaa gcccaaggcc aagcccggca acgcccgcta caccgcgatc 2100
gaggcggcgc ccggccgtta cgtcaaagcc aagggctga 2139

<210> 51
<211> 712
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: (R) - specific
enoyl-CoA transferase Synthase Fusion Protein

<220>
<221> PEPTIDE
<222> (1)..(712)

<400> 51
Met Ser Ala Gln Ser Leu Glu Val Gly Gln Lys Ala Arg Leu Ser Lys
1 5 10 15

Arg Phe Gly Ala Ala Glu Val Ala Ala Phe Ala Ala Leu Ser Glu Asp
20 25 30

Phe Asn Pro Leu His Leu Asp Pro Ala Phe Ala Ala Thr Thr Ala Phe
35 40 45

Glu Arg Pro Ile Val His Gly Met Leu Leu Ala Ser Leu Phe Ser Gly
50 55 60

Leu Leu Gly Gln Gln Leu Pro Gly Lys Gly Ser Ile Tyr Leu Gly Gln
65 70 75 80

Ser Leu Ser Phe Lys Leu Pro Val Phe Val Gly Asp Glu Val Thr Ala
85 90 95

Glu Val Glu Val Thr Ala Leu Arg Glu Asp Lys Pro Ile Ala Thr Leu
100 105 110

Thr Thr Arg Ile Phe Thr Gln Gly Gly Ala Leu Ala Val Thr Gly Glu
115 120 125

Ala Val Val Lys Leu Pro Gly Ser Met Asn Leu Pro Asp Pro Gln Ala
130 135 140

Ile Ala Asn Ala Trp Met Ser Gln Val Gly Asp Pro Ser Gln Trp Gln
145 150 155 160

Ser Trp Phe Ser Lys Ala Pro Thr Thr Glu Ala Asn Pro Met Ala Thr
165 170 175

Met Leu Gln Asp Ile Gly Val Ala Leu Lys Pro Glu Ala Met Glu Gln
180 185 190

Leu Lys Asn Asp Tyr Leu Arg Asp Phe Thr Ala Leu Trp Gln Asp Phe
195 200 205

Leu Ala Gly Lys Ala Pro Ala Val Gln Arg Pro Arg Phe Ser Ser Ala
210 215 220

Ala Trp Gln Gly Asn Pro Met Ser Ala Phe Asn Ala Ala Ser Tyr Leu
225 230 235 240

Leu Asn Ala Lys Phe Leu Ser Ala Met Val Glu Ala Val Asp Thr Ala
245 250 255

Pro Gln Gln Lys Gln Lys Ile Arg Phe Ala Val Gln Gln Val Ile Asp
260 265 270

Ala Met Ser Pro Ala Asn Phe Leu Ala Thr Asn Pro Glu Ala Gln Gln
275 280 285

Lys Leu Ile Glu Thr Lys Gly Glu Ser Leu Thr Arg Gly Leu Val Asn
290 295 300

Met Leu Gly Asp Ile Asn Met Leu Gly Asp Ile Asn Asn Gly His Ile
305 310 315 320

Ser Leu Ser Asp Glu Ser Ala Phe Glu Val Gly Arg Asn Leu Ala Ile
325 330 335

Thr Pro Gly Thr Val Ile Tyr Glu Asn Pro Leu Phe Gln Leu Ile Gln
340 345 350

Tyr Thr Pro Thr Thr Pro Thr Val Ser Gln Arg Pro Leu Leu Met Val
355 360 365

Pro Pro Cys Ile Asn Lys Phe Tyr Ile Leu Asp Leu Gln Pro Glu Asn
370 375 380

Ser Leu Val Arg Tyr Ala Val Glu Gln Gly Asn Thr Val Phe Leu Ile
385 390 395 400

Ser Trp Ser Asn Pro Asp Lys Ser Leu Ala Gly Thr Thr Trp Asp Asp
405 410 415

Tyr Val Glu Gln Gly Val Ile Glu Ala Ile Arg Ile Val Gln Asp Val
420 425 430

Ser Gly Gln Asp Lys Leu Asn Met Phe Gly Phe Cys Val Gly Gly Thr
435 440 445

Ile Val Ala Thr Ala Leu Ala Val Leu Ala Ala Arg Gly Gln His Pro
450 455 460

Ala Ala Ser Leu Thr Leu Leu Thr Thr Phe Leu Asp Phe Ser Asp Thr
465 470 475 480

Gly Cys Ser Thr Ser Cys Arg Glu Thr Gln Val Ala Leu Arg Glu Gln
485 490 495

Gln Leu Arg Asp Gly Gly Leu Met Pro Gly Arg Asp Leu Ala Ser Thr
500 505 510

Phe Ser Ser Leu Arg Pro Asn Asp Leu Val Trp Asn Tyr Val Gln Ser
515 520 525

Asn Tyr Leu Lys Gly Asn Glu Pro Ala Ala Phe Asp Leu Leu Phe Trp
530 535 540

Asn Ser Asp Ser Thr Asn Leu Pro Gly Pro Met Phe Cys Trp Tyr Leu
545 550 555 560

Arg Asn Thr Tyr Leu Glu Asn Ser Leu Lys Val Pro Gly Lys Leu Thr
565 570 575

Val Ala Gly Glu Lys Ile Asp Leu Gly Leu Ile Asp Ala Pro Ala Phe
580 585 590

Ile Tyr Gly Ser Arg Glu Asp His Ile Val Pro Trp Met Ser Ala Tyr
595 600 605

Gly Ser Leu Asp Ile Leu Asn Gln Gly Lys Pro Gly Ala Asn Arg Phe
610 615 620

Val Leu Gly Ala Ser Gly His Ile Ala Gly Val Ile Asn Ser Val Ala
625 630 635 640

Lys Asn Lys Arg Thr Tyr Trp Ile Asn Asp Gly Gly Ala Ala Asp Ala
645 650 655

Gln Ala Trp Phe Asp Gly Ala Gln Glu Val Pro Gly Ser Trp Trp Pro
660 665 670

```
Gln Trp Ala Gly Phe Leu Thr Gln His Gly Gly Lys Lys Val Lys Pro
        675                 680                 685

Lys Ala Lys Pro Gly Asn Ala Arg Tyr Thr Ala Ile Glu Ala Ala Pro
    690                 695                 700

Gly Arg Tyr Val Lys Ala Lys Gly
705                 710
```

<210> 52
<211> 1185
<212> DNA
<213> Aeromonas caviae

<220>
<221> gene
<222> (1)..(1185)
<223> bktB gene

<400> 52
atgacgcgtg aagtggtagt ggtaagcggt gtccgtaccg cgatcgggac ctttggcggc 60
agcctgaagg atgtggcacc ggcggagctg ggcgcactgg tggtgcgcga ggcgctggcg 120
cgcgcgcagg tgtcgggcga cgatgtcggc cacgtggtat tcggcaacgt gatccagacc 180
gagccgcgcg acatgtatct gggccgcgtc gcggccgtca acggcggggt gacgatcaac 240
gcccccgcgc tgaccgtgaa ccgcctgtgc ggctcgggcc tgcaggccat tgtcagcgcc 300
gcgcagacca tcctgctggg cgataccgac gtcgccatcg gcggcggcgc ggaaagcatg 360
agccgcgcac cgtacctggc gccggcagcg cgctggggcg cacgcatggg cgacgccggc 420
ctggtcgaca tgatgctggg tgcgctgcac gatcccttcc atcgcatcca catgggcgtg 480
accgccgaga atgtcgccaa ggaatacgac atctcgcgcg cgcagcagga cgaggccgcg 540
ctggaatcgc accgccgcgc ttcggcagcg atcaaggccg gctacttcaa ggaccagatc 600
gtcccggtgg tgagcaaggg ccgcaagggc gacgtgacct tcgacaccga cgagcacgtg 660
cgccatgacg ccaccatcga cgacatgacc aagctcaggc cggtcttcgt caaggaaaac 720
ggcacggtca cggccggcaa tgcctcgggc ctgaacgacg ccgccgccgc ggtggtgatg 780
atggagcgcg ccgaagccga gcgccgcggc ctgaagccgc tggcccgcct ggtgtcgtac 840
ggccatgccg gcgtggaccc gaaggccatg ggcatcggcc cggtgccggc gacgaagatc 900
gcgctggagc gcgccggcct gcaggtgtcg gacctggacg tgatcgaagc caacgaagcc 960
tttgccgcac aggcgtgcgc cgtgaccaag gcgctcggtc tggacccggc caaggttaac 1020
ccgaacggct cgggcatctc gctgggccac ccgatcggcg ccaccggtgc cctgatcacg 1080
gtgaaggcgc tgcatgagct gaaccgcgtg cagggccgct acgcgctggt gacgatgtgc 1140
atcggcggcg ggcagggcat tgccgccatc ttcgagcgta tctga 1185

<210> 53
<211> 394
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: thiolase II

<220>
<221> PEPTIDE
<222> (1)..(394)

<400> 53
Met Thr Arg Glu Val Val Val Val Ser Gly Val Arg Thr Ala Ile Gly
1   5   10   15

Thr Phe Gly Gly Ser Leu Lys Asp Val Ala Pro Ala Glu Leu Gly Ala
            20   25   30

Leu Val Val Arg Glu Ala Leu Ala Arg Ala Gln Val Ser Gly Asp Asp
        35   40   45

Val Gly His Val Val Phe Gly Asn Val Ile Gln Thr Glu Pro Arg Asp
    50   55   60

Met Tyr Leu Gly Arg Val Ala Ala Val Asn Gly Gly Val Thr Ile Asn
65   70   75   80

Ala Pro Ala Leu Thr Val Asn Arg Leu Cys Gly Ser Gly Leu Gln Ala
                85   90   95

Ile Val Ser Ala Ala Gln Thr Ile Leu Leu Gly Asp Thr Asp Val Ala
            100   105   110

Ile Gly Gly Gly Ala Glu Ser Met Ser Arg Ala Pro Tyr Leu Ala Pro
        115   120   125

Ala Ala Arg Trp Gly Ala Arg Met Gly Asp Ala Gly Leu Val Asp Met
    130   135   140

Met Leu Gly Ala Leu His Asp Pro Phe His Arg Ile His Met Gly Val
145   150   155   160

Thr Ala Glu Asn Val Ala Lys Glu Tyr Asp Ile Ser Arg Ala Gln Gln
                165   170   175

Asp Glu Ala Ala Leu Glu Ser His Arg Arg Ala Ser Ala Ala Ile Lys
            180   185   190

Ala Gly Tyr Phe Lys Asp Gln Ile Val Pro Val Val Ser Lys Gly Arg
        195   200   205

Lys Gly Asp Val Thr Phe Asp Thr Asp Glu His Val Arg His Asp Ala

210 215 220

Thr Ile Asp Asp Met Thr Lys Leu Arg Pro Val Phe Val Lys Glu Asn
225 230 235 240

Gly Thr Val Thr Ala Gly Asn Ala Ser Gly Leu Asn Asp Ala Ala Ala
245 250 255

Ala Val Val Met Met Glu Arg Ala Glu Ala Glu Arg Arg Gly Leu Lys
260 265 270

Pro Leu Ala Arg Leu Val Ser Tyr Gly His Ala Gly Val Asp Pro Lys
275 280 285

Ala Met Gly Ile Gly Pro Val Pro Ala Thr Lys Ile Ala Leu Glu Arg
290 295 300

Ala Gly Leu Gln Val Ser Asp Leu Asp Val Ile Glu Ala Asn Glu Ala
305 310 315 320

Phe Ala Ala Gln Ala Cys Ala Val Thr Lys Ala Leu Gly Leu Asp Pro
325 330 335

Ala Lys Val Asn Pro Asn Gly Ser Gly Ile Ser Leu Gly His Pro Ile
340 345 350

Gly Ala Thr Gly Ala Leu Ile Thr Val Lys Ala Leu His Glu Leu Asn
355 360 365

Arg Val Gln Gly Arg Tyr Ala Leu Val Thr Met Cys Ile Gly Gly Gly
370 375 380

Gln Gly Ile Ala Ala Ile Phe Glu Arg Ile
385 390

<210> 54
<211> 43
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide primer- A1 II up I

<400> 54
ggaattcagg aggttttatg acgcgtgaag tggtagtggt aag 43

<210> 55
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide primer- A1-II up II

<400> 55
cgggatccga tacgctcgaa gatggcggc 29

<210> 56
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide primer- A1-II dw I

<400> 56
cgggatccac gcgtgaagtg gtagtggtaa g 31

<210> 57
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide primer- A1-II dw II

<400> 57
gctctagaag ctttcagata cgctcgaaga tggcggc 37

<210> 58
<211> 1929
<212> DNA
<213> Ralstonia eutropha

<220>
<221> gene
<222> (1)..(1929)
<223> bktB-linker-phbB fusion gene

<400> 58
atgacgcgtg aagtggtagt ggtaagcggt gtccgtaccg cgatcgggac ctttggcggc 60

agcctgaagg atgtggcacc ggcggagctg ggcgcactgg tggtgcgcga ggcgctggcg 120
cgcgcgcagg tgtcgggcga cgatgtcggc cacgtggtat tcggcaacgt gatccagacc 180
gagccgcgcg acatgtatct gggccgcgtc gcggccgtca acggcggggt gacgatcaac 240
gcccccgcgc tgaccgtgaa ccgcctgtgc ggctcgggcc tgcaggccat tgtcagcgcc 300
gcgcagacca tcctgctggg cgataccgac gtcgccatcg gcggcggcgc ggaaagcatg 360
agccgcgcac cgtacctggc gccggcagcg cgctggggcg cacgcatggg cgacgccggc 420
ctggtcgaca tgatgctggg tgcgctgcac gatcccttcc atcgcatcca catgggcgtg 480
accgccgaga atgtcgccaa ggaatacgac atctcgcgcg cgcagcagga cgaggccgcg 540
ctggaatcgc accgccgcgc ttcggcagcg atcaaggccg gctacttcaa ggaccagatc 600
gtcccggtgg tgagcaaggg ccgcaagggc gacgtgacct tcgacaccga cgagcacgtg 660
cgccatgacg ccaccatcga cgacatgacc aagctcaggc cggtcttcgt caaggaaaac 720
ggcacggtca cggccggcaa tgcctcgggc ctgaacgacg ccgccgccgc ggtggtgatg 780
atggagcgcg ccgaagccga gcgccgcggc ctgaagccgc tggcccgcct ggtgtcgtac 840
ggccatgccg gcgtggaccc gaaggccatg ggcatcggcc cggtgccggc gacgaagatc 900
gcgctggagc gcgccggcct gcaggtgtcg gacctggacg tgatcgaagc caacgaagcc 960
tttgccgcac aggcgtgcgc cgtgaccaag gcgctcggtc tggacccggc caaggttaac 1020
ccgaacggct cgggcatctc gctgggccac ccgatcggcg ccaccggtgc cctgatcacg 1080
gtgaaggcgc tgcatgagct gaaccgcgtg cagggccgct acgcgctggt gacgatgtgc 1140
atcggcggcg ggcagggcat tgccgccatc ttcgagcgta tcggatccat gactcagcgc 1200
attgcgtatg tgaccggcgg catgggtggt atcggaaccg ccatttgcca gcggctggcc 1260
aaggatggct ttcgtgtggt ggccggttgc ggccccaact cgccgcgccg cgaaaagtgg 1320
ctggagcagc agaaggccct gggcttcgat ttcattgcct cggaaggcaa tgtggctgac 1380
tgggactcga ccaagaccgc attcgacaag gtcaagtccg aggtcggcga ggttgatgtg 1440
ctgatcaaca acgccggtat cacccgcgac gtggtgttcc gcaagatgac ccgcgccgac 1500
tgggatgcgg tgatcgacac caacctgacc tcgctgttca acgtcaccaa gcaggtgatc 1560
gacggcatgg ccgaccgtgg ctggggccgc atcgtcaaca tctcgtcggt gaacgggcag 1620
aagggccagt tcggccagac caactactcc accgccaagg ccggcctgca tggcttcacc 1680
atggcactgg cgcaggaagt ggcgaccaag ggcgtgaccg tcaacacggt ctctccgggc 1740
tatatcgcca ccgacatggt caaggcgatc cgccaggacg tgctcgacaa gatcgtcgcg 1800
acgatcccgg tcaagcgcct gggcctgccg gaagagatcg cctcgatctg cgcctggttg 1860
tcgtcggagg agtccggttt ctcgaccggc gccgacttct cgctcaacgg cggcctgcat 1920
atgggctga 1929

<210> 59
<211> 642
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Thiolase II
Reductase Fusion Protein

<400> 59
Met Thr Arg Glu Val Val Val Val Ser Gly Val Arg Thr Ala Ile Gly
1 5 10 15

Thr Phe Gly Gly Ser Leu Lys Asp Val Ala Pro Ala Glu Leu Gly Ala
20 25 30

Leu Val Val Arg Glu Ala Leu Ala Arg Ala Gln Val Ser Gly Asp Asp
35 40 45

Val Gly His Val Val Phe Gly Asn Val Ile Gln Thr Glu Pro Arg Asp
50 55 60

Met Tyr Leu Gly Arg Val Ala Ala Val Asn Gly Gly Val Thr Ile Asn
65 70 75 80

Ala Pro Ala Leu Thr Val Asn Arg Leu Cys Gly Ser Gly Leu Gln Ala
85 90 95

Ile Val Ser Ala Ala Gln Thr Ile Leu Leu Gly Asp Thr Asp Val Ala
100 105 110

Ile Gly Gly Gly Ala Glu Ser Met Ser Arg Ala Pro Tyr Leu Ala Pro
115 120 125

Ala Ala Arg Trp Gly Ala Arg Met Gly Asp Ala Gly Leu Val Asp Met
130 135 140

Met Leu Gly Ala Leu His Asp Pro Phe His Arg Ile His Met Gly Val
145 150 155 160

Thr Ala Glu Asn Val Ala Lys Glu Tyr Asp Ile Ser Arg Ala Gln Gln
165 170 175

Asp Glu Ala Ala Leu Glu Ser His Arg Arg Ala Ser Ala Ala Ile Lys
180 185 190

Ala Gly Tyr Phe Lys Asp Gln Ile Val Pro Val Val Ser Lys Gly Arg
195 200 205

Lys Gly Asp Val Thr Phe Asp Thr Asp Glu His Val Arg His Asp Ala
210 215 220

Thr Ile Asp Asp Met Thr Lys Leu Arg Pro Val Phe Val Lys Glu Asn
225 230 235 240

Gly Thr Val Thr Ala Gly Asn Ala Ser Gly Leu Asn Asp Ala Ala Ala
245 250 255

Ala Val Val Met Met Glu Arg Ala Glu Ala Glu Arg Arg Gly Leu Lys
260 265 270

Pro Leu Ala Arg Leu Val Ser Tyr Gly His Ala Gly Val Asp Pro Lys
275 280 285

Ala Met Gly Ile Gly Pro Val Pro Ala Thr Lys Ile Ala Leu Glu Arg
290 295 300

Ala Gly Leu Gln Val Ser Asp Leu Asp Val Ile Glu Ala Asn Glu Ala
305 310 315 320

Phe Ala Ala Gln Ala Cys Ala Val Thr Lys Ala Leu Gly Leu Asp Pro
325 330 335

Ala Lys Val Asn Pro Asn Gly Ser Gly Ile Ser Leu Gly His Pro Ile
340 345 350

Gly Ala Thr Gly Ala Leu Ile Thr Val Lys Ala Leu His Glu Leu Asn
355 360 365

Arg Val Gln Gly Arg Tyr Ala Leu Val Thr Met Cys Ile Gly Gly Gly
370 375 380

Gln Gly Ile Ala Ala Ile Phe Glu Arg Ile Gly Ser Met Thr Gln Arg
385 390 395 400

Ile Ala Tyr Val Thr Gly Gly Met Gly Gly Ile Gly Thr Ala Ile Cys
405 410 415

Gln Arg Leu Ala Lys Asp Gly Phe Arg Val Val Ala Gly Cys Gly Pro
420 425 430

Asn Ser Pro Arg Arg Glu Lys Trp Leu Glu Gln Gln Lys Ala Leu Gly
435 440 445

Phe Asp Phe Ile Ala Ser Glu Gly Asn Val Ala Asp Trp Asp Ser Thr
450 455 460

Lys Thr Ala Phe Asp Lys Val Lys Ser Glu Val Gly Glu Val Asp Val
465 470 475 480

Leu Ile Asn Asn Ala Gly Ile Thr Arg Asp Val Val Phe Arg Lys Met
485 490 495

Thr Arg Ala Asp Trp Asp Ala Val Ile Asp Thr Asn Leu Thr Ser Leu
500 505 510

Phe Asn Val Thr Lys Gln Val Ile Asp Gly Met Ala Asp Arg Gly Trp
515 520 525

Gly Arg Ile Val Asn Ile Ser Ser Val Asn Gly Gln Lys Gly Gln Phe
530 535 540

Gly Gln Thr Asn Tyr Ser Thr Ala Lys Ala Gly Leu His Gly Phe Thr
545 550 555 560

Met Ala Leu Ala Gln Glu Val Ala Thr Lys Gly Val Thr Val Asn Thr
565 570 575

Val Ser Pro Gly Tyr Ile Ala Thr Asp Met Val Lys Ala Ile Arg Gln
580 585 590

Asp Val Leu Asp Lys Ile Val Ala Thr Ile Pro Val Lys Arg Leu Gly
595 600 605

Leu Pro Glu Glu Ile Ala Ser Ile Cys Ala Trp Leu Ser Ser Glu Glu
610 615 620

Ser Gly Phe Ser Thr Gly Ala Asp Phe Ser Leu Asn Gly Gly Leu His
625 630 635 640

Met Gly

<210> 60
<211> 1929
<212> DNA
<213> Ralstonia eutropha

<220>
<221> gene
<222> (1)..(1929)
<223> phbB-linker-bktB fusion gene

<400> 60
atgactcagc gcattgcgta tgtgaccggc ggcatgggtg gtatcggaac cgccatttgc 60
cagcggctgg ccaaggatgg ctttcgtgtg gtggccggtt gcggccccaa ctcgccgcgc 120
cgcgaaaagt ggctggagca gcagaaggcc ctgggcttcg atttcattgc ctcggaaggc 180
aatgtggctg actgggactc gaccaagacc gcattcgaca aggtcaagtc cgaggtcggc 240
gaggttgatg tgctgatcaa caacgccggt atcacccgcg acgtggtgtt ccgcaagatg 300
acccgcgccg actgggatgc ggtgatcgac accaacctga cctcgctgtt caacgtcacc 360
aagcaggtga tcgacggcat ggccgaccgt ggctggggcc gcatcgtcaa catctcgtcg 420
gtgaacgggc agaagggcca gttcggccag accaactact ccaccgccaa ggccggcctg 480
catggcttca ccatggcact ggcgcaggaa gtggcgacca agggcgtgac cgtcaacacg 540
gtctctccgg gctatatcgc caccgacatg gtcaaggcga tccgccagga cgtgctcgac 600
aagatcgtcg cgacgatccc ggtcaagcgc ctgggcctgc cggaagagat cgcctcgatc 660
tgcgcctggt tgtcgtcgga ggagtccggt ttctcgaccg gcgccgactt ctcgctcaac 720
ggcggcctgc atatgggcgg atccatgacg cgtgaagtgg tagtggtaag cggtgtccgt 780
accgcgatcg ggacctttgg cggcagcctg aaggatgtgg caccggcgga gctgggcgca 840

ctggtggtgc gcgaggcgct ggcgcgcgcg caggtgtcgg gcgacgatgt cggccacgtg 900
gtattcggca acgtgatcca gaccgagccg cgcgacatgt atctgggccg cgtcgcggcc 960
gtcaacggcg gggtgacgat caacgccccc gcgctgaccg tgaaccgcct gtgcggctcg 1020
ggcctgcagg ccattgtcag cgccgcgcag accatcctgc tgggcgatac cgacgtcgcc 1080
atcggcggcg gcgcggaaag catgagccgc gcaccgtacc tggcgccggc agcgcgctgg 1140
ggcgcacgca tgggcgacgc cggcctggtc gacatgatgc tgggtgcgct gcacgatccc 1200
ttccatcgca tccacatggg cgtgaccgcc gagaatgtcg ccaaggaata cgacatctcg 1260
cgcgcgcagc aggacgaggc cgcgctggaa tcgcaccgcc gcgcttcggc agcgatcaag 1320
gccggctact tcaaggacca gatcgtcccg gtggtgagca agggccgcaa gggcgacgtg 1380
accttcgaca ccgacgagca cgtgcgccat gacgccacca tcgacgacat gaccaagctc 1440
aggccggtct tcgtcaagga aaacggcacg gtcacggccg gcaatgcctc gggcctgaac 1500
gacgccgccg ccgcggtggt gatgatggag cgcgccgaag ccgagcgccg cggcctgaag 1560
ccgctggccc gcctggtgtc gtacggccat gccggcgtgg acccgaaggc catgggcatc 1620
ggcccggtgc cggcgacgaa gatcgcgctg gagcgcgccg gcctgcaggt gtcggacctg 1680
gacgtgatcg aagccaacga agcctttgcc gcacaggcgt gcgccgtgac caaggcgctc 1740
ggtctggacc cggccaaggt taacccgaac ggctcgggca tctcgctggg ccacccgatc 1800
ggcgccaccg gtgccctgat cacggtgaag gcgctgcatg agctgaaccg cgtgcagggc 1860
cgctacgcgc tggtgacgat gtgcatcggc ggcgggcagg gcattgccgc catcttcgag 1920
cgtatctga 1929

<210> 61
<211> 642
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Reductase
Thiolase II Fusion Protein

<220>
<221> PEPTIDE
<222> (1)..(642)

<400> 61
Met Thr Gln Arg Ile Ala Tyr Val Thr Gly Gly Met Gly Gly Ile Gly
1 5 10 15

Thr Ala Ile Cys Gln Arg Leu Ala Lys Asp Gly Phe Arg Val Val Ala
20 25 30

Gly Cys Gly Pro Asn Ser Pro Arg Arg Glu Lys Trp Leu Glu Gln Gln
35 40 45

Lys Ala Leu Gly Phe Asp Phe Ile Ala Ser Glu Gly Asn Val Ala Asp
50 55 60

Trp Asp Ser Thr Lys Thr Ala Phe Asp Lys Val Lys Ser Glu Val Gly
65 70 75 80

Glu Val Asp Val Leu Ile Asn Asn Ala Gly Ile Thr Arg Asp Val Val
85 90 95

Phe Arg Lys Met Thr Arg Ala Asp Trp Asp Ala Val Ile Asp Thr Asn
100 105 110

Leu Thr Ser Leu Phe Asn Val Thr Lys Gln Val Ile Asp Gly Met Ala
115 120 125

Asp Arg Gly Trp Gly Arg Ile Val Asn Ile Ser Ser Val Asn Gly Gln
130 135 140

Lys Gly Gln Phe Gly Gln Thr Asn Tyr Ser Thr Ala Lys Ala Gly Leu
145 150 155 160

His Gly Phe Thr Met Ala Leu Ala Gln Glu Val Ala Thr Lys Gly Val
165 170 175

Thr Val Asn Thr Val Ser Pro Gly Tyr Ile Ala Thr Asp Met Val Lys
180 185 190

Ala Ile Arg Gln Asp Val Leu Asp Lys Ile Val Ala Thr Ile Pro Val
195 200 205

Lys Arg Leu Gly Leu Pro Glu Glu Ile Ala Ser Ile Cys Ala Trp Leu
210 215 220

Ser Ser Glu Glu Ser Gly Phe Ser Thr Gly Ala Asp Phe Ser Leu Asn
225 230 235 240

Gly Gly Leu His Met Gly Gly Ser Met Thr Arg Glu Val Val Val Val
245 250 255

Ser Gly Val Arg Thr Ala Ile Gly Thr Phe Gly Gly Ser Leu Lys Asp
260 265 270

Val Ala Pro Ala Glu Leu Gly Ala Leu Val Val Arg Glu Ala Leu Ala
275 280 285

Arg Ala Gln Val Ser Gly Asp Asp Val Gly His Val Val Phe Gly Asn
290 295 300

Val Ile Gln Thr Glu Pro Arg Asp Met Tyr Leu Gly Arg Val Ala Ala
305 310 315 320

Val Asn Gly Gly Val Thr Ile Asn Ala Pro Ala Leu Thr Val Asn Arg
325 330 335

Leu Cys Gly Ser Gly Leu Gln Ala Ile Val Ser Ala Ala Gln Thr Ile
340 345 350

Leu Leu Gly Asp Thr Asp Val Ala Ile Gly Gly Gly Ala Glu Ser Met
355 360 365

Ser Arg Ala Pro Tyr Leu Ala Pro Ala Ala Arg Trp Gly Ala Arg Met
370 375 380

Gly Asp Ala Gly Leu Val Asp Met Met Leu Gly Ala Leu His Asp Pro
385 390 395 400

Phe His Arg Ile His Met Gly Val Thr Ala Glu Asn Val Ala Lys Glu
405 410 415

Tyr Asp Ile Ser Arg Ala Gln Gln Asp Glu Ala Ala Leu Glu Ser His
420 425 430

Arg Arg Ala Ser Ala Ala Ile Lys Ala Gly Tyr Phe Lys Asp Gln Ile
435 440 445

Val Pro Val Val Ser Lys Gly Arg Lys Gly Asp Val Thr Phe Asp Thr
450 455 460

Asp Glu His Val Arg His Asp Ala Thr Ile Asp Asp Met Thr Lys Leu
465 470 475 480

Arg Pro Val Phe Val Lys Glu Asn Gly Thr Val Thr Ala Gly Asn Ala
485 490 495

Ser Gly Leu Asn Asp Ala Ala Ala Ala Val Val Met Met Glu Arg Ala
500 505 510

Glu Ala Glu Arg Arg Gly Leu Lys Pro Leu Ala Arg Leu Val Ser Tyr
515 520 525

Gly His Ala Gly Val Asp Pro Lys Ala Met Gly Ile Gly Pro Val Pro
530 535 540

Ala Thr Lys Ile Ala Leu Glu Arg Ala Gly Leu Gln Val Ser Asp Leu
545 550 555 560

Asp Val Ile Glu Ala Asn Glu Ala Phe Ala Ala Gln Ala Cys Ala Val
565 570 575

Thr Lys Ala Leu Gly Leu Asp Pro Ala Lys Val Asn Pro Asn Gly Ser
580 585 590

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Gly Ile Ser Leu Gly His Pro Ile Gly Ala Thr Gly Ala Leu Ile Thr
        595                 600                 605

Val Lys Ala Leu His Glu Leu Asn Arg Val Gln Gly Arg Tyr Ala Leu
    610                 615                 620

Val Thr Met Cys Ile Gly Gly Gly Gln Gly Ile Ala Ala Ile Phe Glu
625                 630                 635                 640

Arg Ile
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